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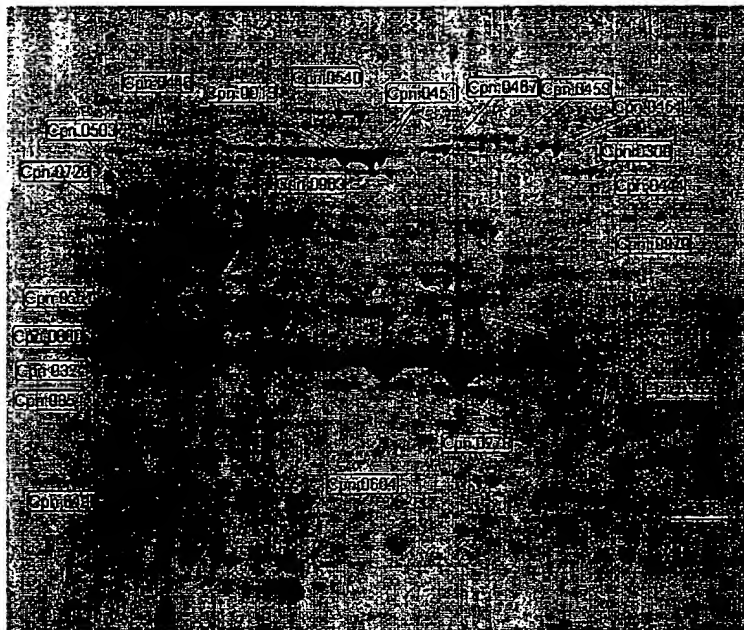
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(54) Title: IMMUNISATION AGAINST *CHLAMYDIA PNEUMONIAE*



(57) Abstract: The published genomic of *Chlamydia pneumoniae* reveals over 1000 putative encoded proteins but does not itself indicate which of these might be useful antigens for immunisation and vaccination or for diagnosis. This difficulty is addressed by the invention, which provides a number of *C. pneumoniae* protein sequences suitable for vaccine production and development and/or for diagnostic purposes.

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IMMUNISATION AGAINST *CHLAMYDIA PNEUMONIAE*

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of immunisation against chlamydial infection, in particular against infection by *Chlamydia pneumoniae*.

BACKGROUND ART

Chlamydiae are obligate intracellular parasites of eukaryotic cells which are responsible for endemic sexually transmitted infections and various other disease syndromes. They occupy an exclusive eubacterial phylogenetic branch, having no close relationship to any other known organisms – they are classified in their own order (*Chlamydiales*) which contains a single family (*Chlamydiaceae*) which in turn contains a single genus (*Chlamydia*). A particular characteristic of the *Chlamydiae* is their unique life cycle, in which the bacterium alternates between two morphologically distinct forms: an extracellular infective form (elementary bodies, EB) and an intracellular non-infective form (reticulate bodies, RB). The life cycle is completed with the re-organization of RB into EB, which subsequently leave the disrupted host cell ready to infect further cells.

Four chlamydial species are currently known – *C.trachomatis*, *C.pneumoniae*, *C.pecorum* and *C.psittaci* [e.g. Raulston (1995) *Mol Microbiol* 15:607-616; Everett (2000) *Vet Microbiol* 75:109-126]. *C.pneumoniae* is closely related to *C.trachomatis*, as the whole genome comparison of at least two isolates from each species has shown [Kalman *et al.* (1999) *Nature Genetics* 21:385-389; Read *et al.* (2000) *Nucleic Acids Res* 28:1397-406; Stephens *et al.* (1998) *Science* 282:754-759]. Based on surface reaction with patient immune sera, the current view is that only one serotype of *C.pneumoniae* exists world-wide.

C.pneumoniae is a common cause of human respiratory disease. It was first isolated from the conjunctiva of a child in Taiwan in 1965, and was established as a major respiratory pathogen in 1983. In the USA, *C.pneumoniae* causes approximately 10% of community-acquired pneumonia and 5% of pharyngitis, bronchitis, and sinusitis.

More recently, the spectrum of *C.pneumoniae* infections has been extended to include atherosclerosis, coronary heart disease, carotid artery stenosis, myocardial infarction, cerebrovascular disease, aortic aneurysm, claudication, and stroke. The association of *C.pneumoniae* with atherosclerosis is corroborated by the presence of the organism in atherosclerotic lesions throughout the arterial tree and the near absence of the organism in healthy arterial tissue. *C.pneumoniae* has also been isolated from coronary and carotid atheromatous plaques. The bacterium has also been associated with other acute and chronic respiratory diseases (e.g. otitis media, chronic obstructive pulmonary disease, pulmonary exacerbation of cystic fibrosis) as a result of sero-epidemiologic observations, case reports, isolation or direct detection of the organism in specimens, and successful

response to anti-chlamydial antibiotics. To determine whether chronic infection plays a role in initiation or progression of disease, intervention studies in humans have been initiated, and animal models of *C.pneumoniae* infection have been developed.

Considerable knowledge of the epidemiology of *C.pneumoniae* infection has been derived from serologic studies using the *C.pneumoniae*-specific microimmunofluorescence test. Infection is ubiquitous, and it is estimated that virtually everyone is infected at some point in life, with common re-infection. Antibodies against *C.pneumoniae* are rare in children under the age of 5, except in developing and tropical countries. Antibody prevalence increases rapidly at ages 5 to 14, reaching 50% at the age of 20, and continuing to increase slowly to ~80% by age 70.

A current hypothesis is that *C.pneumoniae* can persist in an asymptomatic low-grade infection in very large sections of the human population. When this condition occurs, it is believed that the presence of *C.pneumoniae*, and/or the effects of the host reaction to the bacterium, can cause or help progress of cardiovascular illness.

It is not yet clear whether *C.pneumoniae* is actually a causative agent of cardiovascular disease, or whether it is just artefactually associated with it. It has been shown, however, that *C.pneumoniae* infection can induce LDL oxidation by human monocytes [Kalayoglu *et al.* (1999) *J. Infect. Dis.* 180:780-90; Kalayoglu *et al.* (1999) *Am. Heart J.* 138:S488-490]. As LDL oxidation products are highly atherogenic, this observation provides a possible mechanism whereby *C.pneumoniae* may cause atheromatous degeneration. If a causative effect is confirmed, vaccination (prophylactic and therapeutic) will be universally recommended.

Genomic sequence information has been published for *C.pneumoniae* [Kalman *et al.* (1999) *supra*; Read *et al.* (2000) *supra*; Shirai *et al.* (2000) *J. Infect. Dis.* 181(Suppl 3):S524-S527; WO99/27105; WO00/27994] and is available from GenBank. Sequencing efforts have not, however, focused on vaccination, and the availability of genomic sequence does not in itself indicate which of the >1000 genes might encode useful antigens for immunisation and vaccination. WO99/27105, for instance, implies that every one of the 1296 ORFs identified in the *C.pneumoniae* strain CM1 genome is a useful vaccine antigen.

It is thus an object of the present invention to identify antigens useful for vaccine production and development from amongst the many proteins present in *C.pneumoniae*. It is a further object to identify antigens useful for diagnosis (e.g. immunodiagnosis) of *C.pneumoniae*.

DISCLOSURE OF THE INVENTION

The invention provides proteins comprising the *C.pneumoniae* amino acid sequences disclosed in the examples.

It also provides proteins comprising sequences which share at least x% sequence identity with the *C.pneumoniae* amino acid sequences disclosed in the examples. Depending on the particular

sequence, x is preferably 50% or more (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more). These include mutants and allelic variants. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH
5 program (Oxford Molecular), using an affine gap search with parameters *gap open penalty*=12 and *gap extension penalty*=1.

The invention further provides proteins comprising fragments of the *C.pneumoniae* amino acid sequences disclosed in the examples. The fragments should comprise at least n consecutive amino acids from the sequences and, depending on the particular sequence, n is 7 or more (e.g. 8, 10, 12,
10 14, 16, 18, 20, 30, 40, 50, 75, 100 or more). Preferably the fragments comprise one or more epitope(s) from the sequence. Other preferred fragments omit a signal peptide.

The proteins of the invention can, of course, be prepared by various means (e.g. native expression, recombinant expression, purification from cell culture, chemical synthesis *etc.*) and in various forms (e.g. native, fusions *etc.*). They are preferably prepared in substantially pure form (*ie.* substantially
15 free from other *C.pneumoniae* or host cell proteins). Heterologous expression in *E.coli* is a preferred preparative route.

According to a further aspect, the invention provides nucleic acid comprising the *C.pneumoniae* nucleotide sequences disclosed in the examples. In addition, the invention provides nucleic acid comprising sequences which share at least $x\%$ sequence identity with the *C.pneumoniae* nucleotide
20 sequences disclosed in the examples. Depending on the particular sequence, x is preferably 50% or more (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more).

Furthermore, the invention provides nucleic acid which can hybridise to the *C.pneumoniae* nucleic acid disclosed in the examples, preferably under "high stringency" conditions (e.g. 65°C in a 0.1xSSC, 0.5% SDS solution).

25 Nucleic acid comprising fragments of these sequences are also provided. These should comprise at least n consecutive nucleotides from the *C.pneumoniae* sequences and, depending on the particular sequence, n is 10 or more (e.g. 12, 14, 15, 18, 20, 25, 30, 35, 40, 50, 75, 100, 200, 300 or more).

According to a further aspect, the invention provides nucleic acid encoding the proteins and protein fragments of the invention.

30 It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (e.g. for antisense or probing purposes).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g. by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (e.g. single stranded, double stranded, vectors, probes *etc.*).

In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (*e.g.* cloning or expression vectors) and host cells transformed therewith.

- 5 According to a further aspect, the invention provides immunogenic compositions comprising protein and/or nucleic acid according to the invention. These compositions are suitable for immunisation and vaccination purposes. Vaccines of the invention may be prophylactic or therapeutic, and will typically comprise an antigen which can induce antibodies capable of inhibiting (a) chlamydial adhesion, (b) chlamydial entry, and/or (c) successful replication within the host cell. The vaccines
10 preferably induce any cell-mediated T-cell responses which are necessary for chlamydial clearance from the host.

- The invention also provides nucleic acid or protein according to the invention for use as medicaments (*e.g.* as vaccines). It also provides the use of nucleic acid or protein according to the invention in the manufacture of a medicament (*e.g.* a vaccine or an immunogenic composition) for
15 treating or preventing infection due to *C.pneumoniae*.

The invention also provides a method of treating (*e.g.* immunising) a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid or protein according to the invention.

According to further aspects, the invention provides various processes.

- 20 A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression.

A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

- A process for detecting *C.pneumoniae* in a sample is provided, wherein the sample is contacted with
25 an antibody which binds to a protein of the invention.

A summary of standard techniques and procedures which may be employed in order to perform the invention (*e.g.* to utilise the disclosed sequences for immunisation) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

General

- 30 The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature *e.g.* Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989) and *Third Edition* (2001); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I.
35

Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

Definitions

- 10 A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "comprising" means "including" as well as "consisting" e.g. a composition "comprising" X may consist exclusively of X or may include something additional to X, such as X+Y.

- 15 The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Chlamydial sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been
20 assembled in a single protein in an arrangement not found in nature.

- An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be
25 reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

- A "mutant" sequence is defined as DNA, RNA or amino acid sequence differing from but having sequence identity with the native or disclosed sequence. Depending on the particular sequence, the degree of sequence
30 identity between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more, calculated using the Smith-Waterman algorithm as described above). As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs essentially at the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination,
35 has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions (e.g. see US patent 5,753,235).

Expression systems

The Chlamydial nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

i. Mammalian Systems

- 5 Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA
10 synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.].

- 15 Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormone-responsive
20 cells.

- The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription
25 initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.]. Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer [Dijkema et al (1985) *EMBO J.* 4:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al.
30 (1982) *PNAS USA* 79:6777] and from human cytomegalovirus [Boshart et al. (1985) *Cell* 41:521]. Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237].

- A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein
35 will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader

fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

- 5 Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot
- 10 (1989) *Trends Biochem. Sci.* 14:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*].

- Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g. plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing
- 15 the replication systems of papovaviruses, such as SV40 [Gluzman (1981) *Cell* 23:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replicaton systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-
- 20 bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946] and pHEBO [Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074].

- The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion,
- 25 electroporation, encapsulation of polynucleotide(s) in liposomes, direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g. Hep G2), and a number of other cell lines.

35 ii. Baculovirus Systems

- The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site
- 40 for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence

homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its own set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (e.g. plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals

for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human α -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between ~1% and ~5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μ m in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus)

or absence (indicative of recombinant virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers & Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, e.g. Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g. HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g. proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iii. Plant Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wirsal et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, *Gibberellins*: in: *Advanced Plant Physiology*, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Reprtr*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet*, 202:179-185, 1985. The genetic material may also be

transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hieracallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) [Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature* 198:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The *g*-laotamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature* 292:128] and T5 [US patent 4,689,406] promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature* 254:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological*

Regulation and Development: Gene Expression (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*].

5 A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

10 Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai *et al.* (1984) *Nature* 309:810]. Fusion proteins can also be
15 made with sequences from the *lacZ* [Jia *et al.* (1987) *Gene* 60:197], *trpE* [Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11], and *Chey* [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*e.g.* ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign
20 protein. Through this method, native foreign protein can be isolated [Miller *et al.* (1989) *Bio/Technology* 7:698].

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [US patent 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is
25 either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) [Masui *et al.* (1983), in: *Experimental Manipulation of Gene*
30 *Expression*; Ghayeb *et al.* (1984) *EMBO J.* 3:2437] and the *E. coli* alkaline phosphatase signal sequence (*phoA*) [Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 244 042].

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the
35 translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g. plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EP-A- 0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], *Escherichia coli* [Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EP-A-0 036 776, EP-A-0 136 829 and EP-A-0 136 907], *Streptococcus cremoris* [Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655]; *Streptococcus lividans* [Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655], *Streptomyces lividans* [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with CaCl_2 or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See e.g. [Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, *Bacillus*], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; Wang *et al.* (1990) *J. Bacteriol.* 172:949, *Campylobacter*], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. In *Genetic*

Engineering: Proceedings of the International Symposium on Genetic Engineering (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; Escherichia], [Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173 Lactobacillus]; [Fiedler *et al.* (1988) *Anal. Biochem* 170:38, Pseudomonas]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203, Staphylococcus],
 5 [Barany *et al.* (1980) *J. Bacteriol.* 144:698; Harlander (1987) "Transformation of Streptococcus lactis by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Eur. Cong. Biotechnology* 1:412, Streptococcus].

v. Yeast Expression

10 Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may
 15 also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

20 Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences [Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1].

25 In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (US Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the *ADH2*,
 30 *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, [Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119;
 35 Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109;].

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always

be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See *e.g.* EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*e.g.* ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (*e.g.* WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the genes for invertase (EP-A-0012873; JPO 62,096,086) and A-factor (US patent 4,588,684). Alternatively, leaders of non-yeast origin exist, such as an interferon leader, that also provide for secretion in yeast (EP-A-0060057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (US Patents 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (*e.g.* see WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*e.g.* plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YE24 [Botstein *et al.* (1979) *Gene* 8:17-24], pCI/1 [Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646], and YRp17 [Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy

number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See *e.g.* Brake *et al.*, *supra*.

- 5 Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245]. An integrating vector may be
10 directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the
15 expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable
20 marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions [Butt *et al.* (1987) *Microbiol. Rev.* 51:351].

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or
25 developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts: *Candida albicans* [Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142], *Candida maltosa* [Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141], *Hansenula polymorpha* [Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302], *Kluyveromyces fragilis* [Das, *et al.*
30 (1984) *J. Bacteriol.* 158:1165], *Kluyveromyces lactis* [De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135], *Pichia guilliermondii* [Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141], *Pichia pastoris* [Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; US Patent Nos. 4,837,148 and 4,929,555], *Saccharomyces cerevisiae* [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163], *Schizosaccharomyces pombe* [Beach and Nurse (1981) *Nature* 300:706], and *Yarrowia lipolytica* [Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49].

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See *e.g.* [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze
40 *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459;

Roggkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; Hansen *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; Kluyveromyces]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; US Patents 4,837,148 & 4,929,555; Pichia]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach & Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

Pharmaceutical Compositions

Pharmaceutical compositions can comprise polypeptides and/or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

5 Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (e.g. see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

10 Vaccines according to the invention may either be prophylactic (i.e. to prevent infection) or therapeutic (i.e. to treat disease after infection).

Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, 15 polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59TM (WO 90/14837; Chapter 10 in 25 *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RibiTM adjuvant system (RAS), (Ribi Immunochem, Hamilton, 30 MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM); (3) saponin adjuvants, such as StimulonTM (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, 35 such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59TM are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

5 The immunogenic compositions (e.g. the immunising antigen/immunogen/polypeptide/protein/ nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

10 Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

15 Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (e.g. nonhuman primate, primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be
20 determined through routine trials.

The immunogenic compositions are conventionally administered parenterally, e.g. by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (e.g. WO98/20734). Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be
25 administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed [e.g. Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; see later herein].

Gene Delivery Vehicles

30 Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

35 The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses *e.g.* MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (*e.g.* HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No. VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671,

WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include

5 adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the

10 remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (*ie.* there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of

15 which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is

20 SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are

25 herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 & WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those

30 deposited with ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in

35 US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in US Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

40 DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems.

Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Trinita virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70; ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in US 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585.

Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in US 5,149,655; use of ionizing radiation for activating transferred gene, as described in US 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for recombinant protein expression. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*e.g.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in *e.g.* WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

5 Polynucleotide and polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A. Polypeptides

10 One example are polypeptides which include, without limitation: asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

15 B. Hormones, Vitamins, etc.

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, etc.

20 Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

D. Lipids, and Liposomes

25 The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

30 Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See,

also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g. Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E.Lipoproteins

In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, & E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, & E apoproteins, LDL comprises apoprotein B; HDL comprises apoproteins A, C, & E.

The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in

conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750. Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA. Further description of lipoproteins can be found in Zuckermann *et al.* PCT/US97/14465.

10 F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both *in vitro*, *ex vivo*, and *in vivo* applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides/polypeptides.

Nucleic Acid Hybridisation

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook *et al.* [*supra*] vol.2, chapt.9, pp.9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (T_m) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4[\%(G + C)] - 0.6(\% \text{ formamide}) - 600/n - 1.5(\% \text{ mismatch}).$$

where C_i is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (*ie.* stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and

reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Chlamydial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Chlamydial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Chlamydial sequence (or its complement) — some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Chlamydial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Chlamydial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Chlamydial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably ≥ 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* [*J. Am. Chem. Soc.* (1981) 103:3185], or according to Urdea *et al.* [*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461], or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated *e.g.* backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* [*e.g.* see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387]; analogues such as peptide nucleic acids may also be used [*e.g.* see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386].

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* [*Meth. Enzymol.* (1987) 155: 335-350]; US patents 4,683,195 & 4,683,202. Two 'primers' hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its

complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Chlamydial sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labelled probe will hybridize to the Chlamydial sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al* [*supra*]. mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labelled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labelled with a radioactive moiety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-189 show data pertaining to examples 1-189.

Figure 190 shows a representative 2D gel of proteins in elementary bodies.

Figure 191 shows an alignment of sequences in five (six) proteins of the invention.

EXAMPLES

The examples indicate *C.pneumoniae* proteins, together with evidence to support the view that the proteins are useful antigens for vaccine production and development or for diagnostic purposes. This evidence takes the form of:

- Computer prediction based on sequence information from CWL029 strain (*e.g.* using the PSORT algorithm available from www.psорт.nibb.ac.jp).
- Data on recombinant expression and purification of the proteins cloned from IOL207 strain.
- Western blots to demonstrate immunoreactivity in serum (typically a blot of an EB extract of *C.pneumoniae* strain FB/96 stained with mouse antiserum against the recombinant protein).
- FACS analysis of *C.pneumoniae* bacteria or purified EBs to confirm accessibility of the antigen to the immune system (see also table III).
- An indication if the protein was identified by MALDI-TOF from a 2D gel electrophoresis map of proteins from purified elementary bodies from strain FB/96. This confirms that the protein is expressed *in vivo* (see also table V).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins identified in the examples. For example, the proteins can be expressed recombinantly and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question *ie.* the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The recombinant protein can also be conveniently used to prepare antibodies *e.g.* in a mouse. These can be used for direct confirmation that a protein is located on the cell-surface. Labelled antibody (*e.g.* fluorescent labelling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein.

- 5 In particular, the following methods (A) to (O) were used to express, purify and biochemically characterise the proteins of the invention:

CLONING OF CPN ORFs FOR EXPRESSION IN *E. COLI*

ORFs of *Chlamydia pneumoniae* (Cpn) were cloned in such a way as to potentially obtain three different kind of proteins:

- 10 a) proteins having an hexa-histidine tag at the C-terminus (cpn-His)
 b) proteins having a GST fusion partner at the N-terminus (Gst-cpn)
 c) proteins having both hexa-histidine tag at the C-terminus and GST at the N-terminus (GST/His fusion; NH₂-GST-cpn-(His)₆-COOH)

- The type a) proteins were obtained upon cloning in the pET21b+ (Novagen). The type b) and c) proteins were obtained upon cloning in modified pGEX-KG vectors [Guan & Dixon (1991) *Anal. Biochem.* 192:262]. For instance pGEX-KG was modified to obtain pGEX-NN, then by modifying pGEX-NN to obtain pGEX-NNH. The Gst-cpn and Gst-cpn-His proteins were obtained in pGEX-NN and pGEX-NNH respectively.

- The modified versions of pGEX-KG vector were made with the aim of allowing the cloning of single amplification products in all three vectors after only one double restriction enzyme digestion and to minimise the presence of extraneous amino acids in the final recombinant proteins.

(A) Construction of pGEX-NN and pGEX-NNH expression vectors

- Two couples of complementary oligodeoxyribonucleotides were synthesised using the DNA synthesiser ABI394 (Perkin Elmer) and the reagents from Cruachem (Glasgow, Scotland). Equimolar amounts of the oligo pairs (50 ng each oligo) were annealed in T4 DNA ligase buffer (New England Biolabs) for 10 min in a final volume of 50µl and then were left to cool slowly at room temperature. With the described procedure the following DNA linkers were obtained:

gexNN linker:

- 30 NdeI NheI XmaI EcoRI NcoI SalI XhoI SacI NotI
 GATCCCATATGGCTAGCCCCGGGAATTCGTCCATGGAGTGAGTCGACTGACTCGAGTGATCGAGCTCCTGAGCGGCCGCATGAA
 GGTATACCGATCGGGCCCCCTAAGCAGGTACCTCACTCAGCTGACTGAGCTCACTAGCTCGAGGACTCGCCGGCGTACTTTCTGA

gexNNH linker:

- 35 HindIII NotI XhoI --Hexa-Histidine--
 TCGACAAGCTTGCGGCCGCACTCGAGCATCACCATCACCATCACTGAT
 GTTCGAACGCCGGCGTGAGCACGTAGAGGTAGTGGTAGTGACTATCGA

The plasmid pGEX-KG was digested with BamHI and HindIII and 100 ng were ligated overnight at 16 °C to the linker gexNN with a molar ratio of 3:1 linker/plasmid using 200 units of T4 DNA ligase

(New england Biolabs). After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NN plasmid, having the correct linker, was selected by means of restriction enzyme analysis and DNA sequencing.

The new plasmid pGEX-NN was digested with SalI and HindIII and ligated to the linker gexNNH.

- 5 After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NNH plasmid, having the correct linker, was selected by means of restriction enzyme analysis and DNA sequencing.

(B) Chromosomal DNA preparation

- 10 The chromosomal DNA of elementary bodies (EB) of *C.pneumoniae* strain 10L-207 was prepared by adding 1.5 ml of lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 0,6 % SDS, 100 µg/ml Proteinase K, pH 8) to 450 µl EB suspension (400.000/µl) and incubating overnight at 37 °C. After sequential extraction with phenol, phenol-chloroform, and chloroform, the DNA was precipitated with 0,3 M sodium acetate, pH 5,2 and 2 volumes of absolute ethanol. The DNA pellet was washed with 70 % ethanol. After solubilization with distilled water and treatment with 20 µg/ml RNase A
- 15 for 1 hour at RT, the DNA was extracted again with phenol-chloroform, alcohol precipitated and suspended with 300 µl 1 mM Tris-HCl pH 8,5. The DNA concentration was evaluated by measuring OD₂₆₀ of the sample.

(C) Oligonucleotide design

- 20 Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF using the sequence of *C.pneumoniae* strain CWL029. Any predicted signal peptide were omitted, by deducing the 5' end amplification primer sequence immediately downstream from the predicted leader sequence. For most ORFs, the 5' tail of the primers (table I) included only one restriction enzyme recognition site (NdeI, or NheI, or SpeI depending on the gene's own restriction pattern); the 3' primer tails (tableI) included a XhoI or a NotI or a HindIII restriction site.

5' tails		3' tails	
NdeI	5' GTGCGTCATATG 3'	XhoI	5' GCGTCTCGAG 3'
NheI	5' GTGCGTGCTAGC 3'	NotI	5' ACTCGCTAGCGGCCGC 3'
SpeI	5' GTGCGTACTAGT 3'	HindIII	5' GCGTAAGCTT 3'

25 **Table I.** Oligonucleotide tails of the primers used to amplify Cpn genes.

- As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the primers which was determined as described [(Breslauer *et al.* (1986) *PNAS USA* 83:3746-50)]. The average melting temperature of the selected oligos was 50-55°C
- 30 for the hybridizing region alone and 65-75°C for the whole oligos. Table II shows the forward and reverse primers used for each amplification.

(D) Amplification

The standard PCR protocol was as follow: 50 ng genomic DNA were used as template in the presence of 0,2 μ M each primer, 200 μ M each dNTP, 1,5 mM $MgCl_2$, 1x PCR buffer minus Mg (Gibco-BRL), and 2 units of Taq DNA polymerase (Platinum Taq, Gibco-BRL) in a final volume of 100 μ l. Each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridizing temperature the one of the oligos excluding the restriction enzyme tail, followed by 25 cycles performed according to the hybridization temperature of the whole lenght primers. The standard cycles were as follow:

denaturation : 94 °C, 2 min

denaturation: 94 °C, 30 seconds	}	5 cycles
hybridization: 51 °C, 50 seconds		
elongation: 72 °C, 1 min or 2 min and 40 sec		

denaturation: 94 °C, 30 seconds	}	25 cycles
hybridization: 70 °C, 50 seconds		
elongation: 72 °C, 1 min or 2 min and 40 sec		

72 °C, 7 min

4 °C

The elongation time was 1 min for ORFs shorter than 2000 bp, and 2 min and 40 seconds for ORFs longer than 2000 bp. The amplifications were performed using a Gene Amp PCR system 9600 (Perkin Elmer).

To check the amplification results, 4 μ l of each PCR product was loaded onto 1-1.5 agarose gel and the size of amplified fragments compared with DNA molecular weight standards (DNA markers III or IX, Roche). The PCR products were loaded on agarose gel and after electrophoresis the right size bands were excised from the gel. The DNA was purified from the agarose using the Gel Extraction Kit (Qiagen) following the instruction of the manufacturer. The final elution volume of the DNA was 50 μ l TE (10 mM Tris-HCl, 1 mM EDTA, pH 8). One μ l of each purified DNA was loaded onto agarose gel to evaluate the yield.

(E) Digestion of PCR fragments

One-two μ g of purified PCR product were double digested overnight at 37 °C with the appropriate restriction enzymes (60 units of each enzyme) using the appropriate restriction buffer in 100 μ l final volume. The restriction enzymes and the digestion buffers were from New England Biolabs. After

purification of the digested DNA (PCR purification Kit, Qiagen) and elution with 30 µl TE, 1 µl was subjected to agarose gel electrophoresis to evaluate the yield in comparison to titrated molecular weight standards (DNA markers III or IX, Roche).

(F) Digestion of the cloning vectors (pET21b+, pGEX-NN, and pGEX-NNH)

- 5 10 µg of plasmid was double digested with 100 units of each restriction enzyme in 400 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37 °C. After electrophoresis on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen Qiaex II Gel Extraction Kit and the DNA was eluted with 50 µl TE. The DNA concentration was evaluated by measuring OD₂₆₀ of the sample.

10 **(G) Cloning**

75ng of the appropriately digested and purified vectors and the digested and purified fragments corresponding to each ORF, were ligated in final volumes of 10-20 µl with a molar ratio of 1:1 fragment/vector, using 400 units T4 DNA ligase (New England Biolabs) in the presence of the buffer supplied by the manufacturer. The reactions were incubated overnight at 16 °C.

- 15 Transformation in *E. coli* DH5 competent cells was performed as follow: the ligation reaction was mixed with 200 µl of competent DH5 cells and incubated on ice for 30 min and then at 42 °C for 90 seconds. After cooling on ice, 0.8 ml LB was added and the cells were incubated for 45 min at 37 °C under shaking. 100 and 900 µl of cell suspensions were plated on separate plates of agar LB 100 µg/ml Ampicillin and the plates were incubated overnight at 37 °C. The screening of the
- 20 transformants was done by growing randomly chosen clones in 6 ml LB 100 µg/ml Ampicillin, by extracting the DNA using the Qiagen Qiaprep Spin Miniprep Kit following the manufacturer instructions, and by digesting 2 µl of plasmid miniprep with the restriction enzymes specific for the restriction cloning sites. After agarose gel electrophoresis of the digested plasmid mini-preparations, positive clones were chosen on the basis of the correct size of the restriction fragments,
- 25 as evaluated by comparison with appropriate molecular weight markers (DNA markers III or IX, Roche).

(H) Expression

- 1 µl of each right plasmid mini-preparation was transformed in 200 µl of competent *E. coli* strain suitable for expression of the recombinant protein. All pET21b+ recombinant plasmids were
- 30 transformed in BL21 DE3 (Novagen) *E. coli* cells, whilst all pGEX-NN and all pGEX-NNH recombinant plasmids were transformed in BL21 cells (Novagen). After plating transformation mixtures on LB/Amp agar plates and incubation overnight at 37 °C, single colonies were inoculated in 3 ml LB 100 µg/ml Ampicillin and grown at 37 °C overnight. 70 µl of the overnight culture was inoculated in 2 ml LB/Amp and grown at 37 °C until OD₆₀₀ of the pET clones reached the 0,4-0,8
- 35 value or until OD₆₀₀ of the pGEX clones reached the 0,8-1 value. Protein expression was then

induced by adding IPTG (Isopropil β -D thio-galacto-piranoside) to the mini-cultures. pET clones were induced using 1 mM IPTG, whilst pGEX clones were induced using 0.2 mM IPTG. After 3 hours incubation at 37 °C the final OD₆₀₀ was checked and the cultures were cooled on ice. After centrifugation of 0.5 ml culture, the cell pellet was suspended in 50 μ l of protein Loading Sample Buffer (60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% w/v Bromophenol Blue, 100 mM DTT) and incubated at 100 °C for 5 min. A volume of boiled sample corresponding to 0.1 OD₆₀₀ culture was analysed by SDS-PAGE and Coomassie Blue staining to verify the presence of induced protein band.

PURIFICATION OF THE RECOMBINANT PROTEINS

- Single colonies were inoculated in 25 ml LB 100 μ g/ml Ampicillin and grown at 37 °C overnight. The overnight culture was inoculated in 500 ml LB/Amp and grown under shaking at 25 °C until OD₆₀₀ 0,4-0,8 value for the pET clones, or until OD₆₀₀ 0,8-1 value for the pGEX clones. Protein expression was then induced by adding IPTG to the cultures. pET clones were induced using 1 mM IPTG, whilst pGEX clones were induced using 0.2 mM IPTG. After 4 hours incubation at 25 °C the final OD₆₀₀ was checked and the cultures were cooled on ice. After centrifugation at 6000 rpm (JA10 rotor, Beckman), the cell pellet was processed for purification or frozen at -20 °C.

(I) Procedure for the purification of soluble His-tagged proteins from *E.coli*

1. Transfer the pellets from -20°C to ice bath and reconstitute with 10 ml 50 mM NaHPO₄ buffer, 300 mM NaCl, pH 8,0, pass in 40-50 ml centrifugation tubes and break the cells as per the following outline:
2. Break the pellets in the French Press performing three passages with in-line washing.
3. Centrifuge at about 30-40000 x g per 15-20 min. If possible use rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.)
4. Equilibrate the Poly-Prep columns with 1 ml Fast Flow Chelating Sepharose resin with 50 mM phosphate buffer, 300 mM NaCl, pH 8,0.
5. Store the centrifugation pellet at -20°C, and load the supernatant in the columns.
6. Collect the flow through.
7. Wash the columns with 10 ml (2 ml + 2 ml + 4 ml) 50 mM phosphate buffer, 300 mM NaCl, pH 8,0.
8. Wash again with 10 ml 20 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8,0.
9. Elute the proteins bound to the columns with 4,5 ml (1,5 ml + 1,5 ml + 1,5 ml) 250 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8,0 and collect the 3 corresponding fractions of ~1,5 ml each. Add to each tube 15 μ l DTT 200 mM (final concentration 2 mM)

10. Measure the protein concentration of the first two fractions with the Bradford method, collect a 10 µg aliquot of proteins from each sample and analyse by SDS-PAGE. (N.B.: should the sample be too diluted, load 21 µl + 7 µl loading buffer).
11. Store the collected fractions at +4°C while waiting for the results of the SDS-PAGE analysis.
- 5 12. For immunisation prepare 4-5 aliquots of 100 µg each in 0,5 ml in 40% glycerol. The dilution buffer is the above elution buffer, plus 2 mM DTT. Store the aliquots at -20°C until immunisation.

(J) Purification of His-tagged proteins from Inclusion bodies

Purifications were carried out essentially according the following protocol:

- 10 1. Bacteria are collected from 500 ml cultures by centrifugation. If required store bacterial pellets at -20°C. For extraction, resuspend each bacterial pellet in 10 ml 50 mM TRIS-HCl buffer, pH 8,5 on an ice bath.
2. Disrupt the resuspended bacteria with a French Press, performing two passages.
3. Centrifuge at 35000 x g for 15 min and collect the pellets. Use a Beckman rotor JA 25.50 (21000
15 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.).
4. Dissolve the centrifugation pellets with 50 mM TRIS-HCl, 1 mM TCEP {Tris(2-carboxyethyl)-phosphine hydrochloride, Pierce} , 6M guanidium chloride, pH 8,5. Stir for ~ 10 min. with a magnetic bar.
5. Centrifuge as described above, and collect the supernatant..
- 20 6. Prepare an adequate number of Poly-Prep (Bio-Rad) columns containing 1 ml of Fast Flow Chelating Sepharose (Pharmacia) saturated with Nichel according to manufacturer recommendations.. Wash the columns twice with 5 ml of H₂O and equilibrate with 50 mM TRIS-HCl, 1 mM TCEP, 6M guanidinium chloride, pH 8,5.
7. Load the supernatants from step 5 onto the columns, and wash with 5 ml of 50 mM TRIS-Hcl
25 buffer, 1 mM TCEP, 6M urea, pH 8,5
8. Wash the columns with 10 ml of 20 mM imidazole, 50 mM TRIS-HCl , 6M urea, 1 mM TCEP, pH 8,5. Collect and set aside the first 5 ml for possible further controls.
9. Elute the proteins bound to the columns with 4,5 ml of a buffer containing 250 mM imidazole, 50 mM TRIS-HCl, 6M urea, 1 mM TCEP, pH 8,5. Add the elution buffer in three 1,5 ml aliquots,
30 and collect the corresponding 3 fractions. Add to each fraction 15 µl DTT (final concentration 2 mM) .
10. Measure eluted protein concentration with the Bradford method, and analyze aliquots of ca 10 µg of protein by SDS-PAGE.
11. Store proteins at -20°C in 40% (v/v) glycerol, 50 mM TRIS-HCl, 2M urea, 0.5 M arginine, 2 mM
35 DTT, 0.3 mM TCEP, 83.3 mM imidazole, pH 8,5

(K) Procedure for the purification of GST-fusion proteins from *E.coli*

1. Transfer the bacterial pellets from -20°C to an ice bath and resuspend with 7,5 ml PBS, pH 7,4 to which a mixture of protease inhibitors (CØMPLETE™ - Boehringer Mannheim, 1 tablet every 25 ml of buffer) has been added. Transfer to 40-50 ml centrifugation tubes and sonicate according to the following procedure:
 - a) Position the probe at about 0,5 cm from the bottom of the tube
 - b) Block the tube with the clamp
 - c) Dip the tube in an ice bath
 - d) Set the sonicator as follows: Timer \rightarrow Hold, Duty Cycle \rightarrow 55, Out. Control \rightarrow 6.
 - e) perform 5 cycles of 10 impulses at a time lapse of 1 minute (i.e. one cycle = 10 impulses + ~45" hold; b. 10 impulses + ~45" hold; c. 10 impulses + ~45" hold; d. 10 impulses + ~45" hold; e. 10 impulses + ~45" hold)
2. Centrifuge at about $30-40000 \times g$ for 15-20 min. E.g.: use rotor Beckman JA 25.50 at 21000 rpm, for 15 min.
3. Store the centrifugation pellets at -20°C , and load the supernatants on the chromatography columns, as follows
4. Equilibrate the Poly-Prep (Bio-Rad) columns with 0,5 ml (\cong 1 ml suspension) of Glutathione-Sepharose 4B resin, wash with 2 ml (1 + 1) H_2O , and then with 10 ml (2 + 4 + 4) PBS, pH 7,4.
5. Load the supernatants on the columns and discard the flow through.
6. Wash the columns with 10 ml (2 + 4 + 4) PBS, pH 7,4.
7. Elute the proteins bound to the columns with 4,5 ml of 50 mM TRIS buffer, 10 mM reduced glutathione, pH 8,0, adding 1,5 ml + 1,5 ml + 1,5 ml and collecting the respective 3 fractions of ~1,5 ml each.
8. Measure the protein concentration of the first two fractions with the Bradford method, analyse a 10 μg aliquot of proteins from each sample by SDS-PAGE. (N.B.: if the sample is too diluted load 21 μl (+ 7 μl loading buffer).
9. Store the collected fractions at $+4^{\circ}\text{C}$ while waiting for the results of the SDS-PAGE analysis.
10. For each protein destined to the immunisation prepare 4-5 aliquots of 100 μg each in 0,5 ml of 40% glycerol. The dilution buffer is 50 mM TRIS.HCl, 2 mM DTT, pH 8,0. Store the aliquots at -20°C until immunisation..

SEROLOGY**(L) Protocol of immunization**

1. Groups of four CD1 female mice aged between 6 and 7 weeks were immunized with 20 μg of recombinant protein resuspended in 100 μl .

2. Four mice for each group received 3 doses with a 14 days interval schedule.
3. Immunization was performed through intra-peritoneal injection of the protein with an equal volume of Complete Freund's Adjuvant (CFA) for the first dose and Incomplete Freund's Adjuvant (IFA) for the following two doses.
- 5 4. Sera were collected before each immunization. Mice were sacrificed 14 days after the third immunization and the collected sera were pooled and stored at -20°C .

(M) Western blot analysis of Cpn elementary body proteins with mouse sera

- Aliquots of elementary bodies containing approximately 4 μg of proteins, mixed with SDS loading buffer (1x: 60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% Bromophenol Blue, 100 mM DTT) and boiled 5 minutes at 95°C , were loaded on a 12% SDS-PAGE gel. The gel was run using a SDS-PAGE running buffer containing 250 mM TRIS, 2.5 mM Glycine and 0.1 %SDS. The gel was electroblotted onto nitrocellulose membrane at 200 mA for 30 minutes. The membrane was blocked for 30 minutes with PBS, 3% skimmed milk powder and incubated O/N at 4°C with the appropriate dilution (1/100) of the sera. After washing twice with PBS + 0.1% Tween (Sigma) the membrane was incubated for 2 hours with peroxidase-conjugated secondary anti-mouse antibody (Sigma) diluted 1:3000. The nitrocellulose was washed twice for 10 minutes with PBS + 0.1% Tween-20 and once with PBS and thereafter developed by Opti-4CN Substrate Kit (Biorad).

Lanes shown in Western blots are: (P) = pre-immune control serum; (I) = immune serum.

(N) FACS analysis of *Chlamydia pneumoniae* elementary bodies with mouse sera

- 20 1. 2×10^5 Elementary Bodies (EB)/well were washed with 200 μl of PBS-0.1%BSA in a 96 wells U bottom plate and centrifuged for 10 min. at 1200rpm, at 4°C .
2. The supernatant was discarded and the E.B. resuspended in 10 μl of PBS-0.1%BSA.
3. 10 μl mouse sera diluted in PBS-0.1%BSA were added to the E.B. suspension to a final dilution of 1:400, and incubated on ice for 30 min.
- 25 4. EB were washed by adding 180 μl PBS-0.1%BSA and centrifuged for 10min. at 1200rpm, 4°C .
5. The supernatant was discarded and the E.B. resuspended in 10 l of PBS-0.1%BSA.
6. 10 μl of a goat anti-mouse IgG, F(ab')₂ fragment specific-R-Phycoerythrin-conjugated (Jackson ImmunoResearch Laboratories Inc., cat.N°115-116-072) was added to the EB suspension to a final dilution of 1:100, and incubated on ice for 30 min. in the dark.
- 30 7. EB were washed by adding 180 μl PBS-0.1%BSA and centrifuged for 10min. at 1200rpm, 4°C .
8. The supernatant was discarded and the E.B. resuspended in 150 μl of PBS-0.1%BSA.
9. E.B. suspension was passed through a cytometric chamber of a FACS Calibur (Becton Dickinson, Mountain View, CA USA) and 10.000 events were acquired.

10. Data were analysed using Cell Quest Software (Becton Dickinson, Mountain View, CA USA) by drawing a morphological dot plot (using forward and side scatter parameters) on E.B. signals. An histogram plot was then created on FL2 intensity of fluorescence log scale recalling the morphological region of EB.

- 5 NB: the results of FACS depend not only on the extent of accessibility of the native antigens but also on the quality of the antibodies elicited by the recombinant antigens, which may have structures with a variable degree of correct folding as compared with the native protein structures. Therefore, even if a FACS assay appears negative this does not necessarily mean that the protein is not abundant or accessible on the surface. PorB antigen, for instance, gave negative results in FACS but is a surface-exposed neutralising antigen [Kubo & Stephens (2000) *Mol. Microbiol.* 38:772-780].

(O) Mass Spectrometry analysis of two-dimensional electrophoretic protein maps

- Gradient purified EBs from strain FB/96 were solubilized at a final concentration of 5.5mg/ml with immobiline rehydration buffer (7M urea, 2M thiourea, 2% (w/v) CHAPS, 2% (w/v) ASB 14 [Chevallet *et al.* (1998) *Electrophor.* 19:1901-9], 2% (v/v) C.A 3-10NL (Amersham Pharmacia Biotech), 2 mM tributyl phosphine, 65 mM DTT). Samples (250µg protein) were adsorbed overnight on Immobiline DryStrips (7 cm, pH 3-10 non linear). Electrophocusing was performed in a IPGphor Isoelectric Focusing Unit (Amersham Pharmacia Biotech). Before PAGE separation, the focused strips were incubated in 4M urea, 2M thiourea, 30% (v/v) glycerol, 2% (w/v) SDS, 5mM tributyl phosphine 2.5%(w/v) acrylamide, 50mM Tris-HCl pH 8.8, as described [Herbert *et al.* (1998) *Electrophor.* 19:845-51]. SDS-PAGE was performed on linear 9-16% acrylamide gradients. Gels were stained with colloidal Coomassie (Novex, San Diego) [Doherty *et al.* (1998) *Electrophor.* 19:355-63]. Stained gels were scanned with a Personal Densitometer SI (Molecular Dynamics) at 8 bits and 50µm per pixel. Map images were annotated with the software Image Master 2D Elite, version 3.10 (Amersham Pharmacia Biotech). Protein spots were excised from the gel, using an Ettan Spot picker (Amersham Pharmacia Biotech), and dried in a vacuum centrifuge. In-gel digestion of samples for mass spectrometry and extraction of peptides were performed as described by Wilm *et al.* [Nature (1996) 379:466-9]. Samples were desalted with a ZIP TIP (Millipore), eluted with a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 0.1% TFA and directly loaded onto a SCOUT 381 multiprobe plate (Bruker). Spectra were acquired on a Bruker Biflex II MALDI-TOF. Spectra were calibrated using a combination of known standard peptides, located in spots adjacent to the samples. Resulting values for monoisotopic peaks were used for database searches using the computer program Mascot (www.matrixscience.com). All searches were performed using an error of 200-500ppm as constraint. A representative gel is shown in Figure 190.

Example 1

- 35 The following *C.pneumoniae* protein (PID 4376552) was expressed <SEQ ID 1; cp6552>:

1 MKKKLSLLVG LIFVLSSCHK EDQNKIRIV ASPTPHAELL ESLQEEAKDL

51 GIKLKILPVD DYRIPNRLLL DKQVDANYFQ HQAFLLDDECE RYDCKGELVV
 101 IAKVHLEPQA IYSKKHSSLE RLKSQKKLTI AIPVDRTNAQ RALHLLLEECG
 151 LIVCKGPANL NMTAKDVCGK ENRSINILEV SAPLLVGSPL DVDAAVIPGN
 201 FAIAANLSPK KDSLCLLEDLS VSKYTNLVVI RSEDVGSPEM IKLQKLFQSP
 251 SVQHFFDTRY HGNILMTQD NG*

A predicted signal peptide is highlighted.

The cp6552 nucleotide sequence <SEQ ID 2> is:

1 ATGAAAAAAA AATTATCATT ACTTGTAGGT TTAATTTTTG TTTTGAGTTC
 51 TTGCCATAAG GAAGATGCTC AGAATAAAAT ACGTATTGTA GCCAGTCCGA
 101 CACCTCATGC GGAATTATTG GAGAGTTTAC AGGAAGAGGC TAAAGATCTT
 151 GGAATCAAGC TGAAATACT TCCAGTAGAT GATTATCGTA TTCCTAATCG
 201 TTTGCTTTTTG GATAAACAAG TAGATGCAAA TTACTTTCAA CATCAAGCTT
 251 TTCTTGATGA CGAATGCGAG CGTTATGATT GTAAGGGTGA ATTAGTTGTT
 301 ATCGCTAAAG TTCATTTGGA ACCTCAAGCA ATTTATTCTA AGAAACATTC
 15 351 TTCTTTAGAG CGCTTAAAAA GCCAGAAGAA ACTGACTATA GCGATTCCCTG
 401 TGGATCGTAC GAATGCTCAG CGTGCTCTAC ACTTGTTAGA AGAGTGCGGA
 451 CTCATTGTTT GCAAAGGGCC TGCTAATTTA AATATGACAG CTAAAGATGT
 501 CTGTGGGAAA GAAAATAGAA GTATCAACAT ATTAGAGGTG TCAGCTCCTC
 551 TTCTTGTCGG ATCTCTTCCT GACGTTGATG CTGCTGTCAT TCCTGGAAAT
 20 601 TTTGCTATAG CAGCAAACCT TTCTCCAAAG AAAGATAGTC TTTGTTTAGA
 651 GGATCTTTTC GTATCTAAGT ATACAAACCT TGTGTTCATT CGTTCTGAAG
 701 ACGTAGGTTT TCCTAAAATG ATAAAATTAC AGAAGCTGTT TCAATCTCCT
 751 TCTGTACAAC ATTTTTTTGA TACAAAATAT CATGGGAATA TTTTGACAAT
 801 GACTCAAGAC AATGGTTAG

25 The PSORT algorithm predicts an inner membrane location (0.127).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 1A, and also as a GST-fusion. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 1B) and for FACS analysis (Figure 1C).

The cp6552 protein was also identified in the 2D-PAGE experiment (Cpn0278).

30 These experiments show that cp6552 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 2

The following *C.pneumoniae* protein (PID 4376736) was expressed <SEQ ID 3; cp6736>:

1 MKTSIRKFLI SFTLAPCFAS TAFTVEVIMP SENFDGSSGK IFPYTTLSDP
 35 51 RGTLCIFSGD LYIANLDNAI SRTSSSCFSN RAGALQILGK GGVFSFLNIR
 101 SSADGAAISS VITQNPCLP LSFSGFSQMI FDNCESLTSD TSASNVIPHA
 151 SAIYATTPML FTNNDLILFQ YNRSAGFGAA IRGTSITIEN TKKSLLFNNG
 201 GSIISNGGALT GSAAINLINN SAPVIFSTNA TGIYGGATYL TGGSMILTSGN
 251 LSGVLVFNNS SRSGGAIYAN GNVTFPSNNSD LTFQNTASP QNSLPAPTTP
 40 301 PTPPAVTPPL GYGGAIFCTP PATPPPTGVS LTISGENSVT FLENIASEQG
 351 GALYGGKISI DSNKSTIFLG NTAGKGAIA IPESGELSLS ANQGDILFNK
 401 NLSITSGTPT RNSIHFGKDA KFATLGATQG YTLVYFDPIT SDDLSAASAA
 451 ATVVVNPKAS ADGAYSGETIV FSGETLTATE AATPANATST LNQKLELEGG
 501 TLALRNGATL NVHNFTQDEK SVVIMDAGTT LATTNGANNT DGATTLNKLIV
 45 551 INLDSLDTGK AAVNVQSTN GALTISGTLG LVKNSQDCCD NHGMFNKDLQ
 601 QVPILKELKAT SNTVTMTDFS LGTNGYQOSP YGYQGTWEFT IDTTTHTVTG
 651 NWKTGTYLPH PERLAPLIPN SLWANVIDLR AVSQASAADG EDVPGKQLSI
 701 TGITNFFHAN HTGDARSYRH MGGGYLINTY TRITPDAAALS LGFGQLFTKS
 751 KDYLVGHHGS NVYFATVYSN ITKSLFGSSR FFSGTSRVT YSRSNEKVKT
 50 801 SYTKLPKGRG SWSNNCWLGE LEGNLPITLS SRILNLKQII PFVKADEVAYA
 851 THGGIQENTP EGRIFGHGHL LNVAVFVGVF FGKNSHNRPD FYTIIVAYAP
 901 DVYRHNPDCE TTPPINGATW TSIGNNLTRS TLLVQASSHT SVNDVLEIFG
 951 HCGCDIRRTS RQYTLDIGSK LRF*

A predicted signal peptide is highlighted.

The cp6736 nucleotide sequence <SEQ ID 4> is:

```

1  ATGAAACGT CTATTCGTAA GTTCTTAATT TCTACCACAC TGGCGCCATG
51  TTTTGGCTTCA ACAGCGTTTA CTGTAGAAGT TATCATGCCT TCCGAGAACT
101  TTGATGGATC GAGTGGGAAG ATTTTTCCTT ACACAACACT TTCTGATCCT
5   151  AGAGGGACAC TCTGTATTTT TTCAGGGGAT CTCTACATTG CGAATCTTGA
201  TAATGCCATA TCCAGAACCT CTTCAGTTG CTTTAGCAAT AGGGCGGGAG
251  CACTACAAAT CTTAGGAAAA GGTGGGGTTT TCTCCTTCTT AAATATCCGT
301  TCTTCAGCTG ACGGAGCCGC GATTAGTAGT GTAATCACCC AAAATCCTGA
10  351  ACTATGTCCC TTGAGTTTTT CAGGATTTAG TCAGATGATC TTCGATAACT
401  GTGAATCTTT GACTTCAGAT ACCTCAGCGA GTAATGTCAT ACCTCAGCGA
451  TCGGCGATTT ACGCTACAAC GCCCATGCTC TTTACAAACA ATGACTCCAT
501  ACTATTCCAA TACAACCGTT CTGCAGGATT TGGAGCTGCC ATTCGAGGCA
551  CAAGCATCAC AATAGAAAAA ACGAAAAAGA GCCTTCTCTT TAATGGTAAT
601  GGATCCATCT CTAATGGAGG GGCCCTCAGC GGATCTGCAG CGATCAACCT
15  651  CATCAACAAT AGCGCTCCTG TGATTTTCTC AACGAATGCT ACAGGGATCT
701  ATGGTGGGCG TATTACCTT ACCGGAGGAT CTATGCTCAC CTCTGGGAAC
751  CTCTCAGGAG TCTTGTTCTG TAATAATAGC TCGCGCTCAG GAGGCGCTAT
801  CTATGCTAAC GGAAATGTCA CATTTTCTAA TAACAGCGAC CTGACTTFFC
851  AAAACAATAC AGCATCTCCA CAAAACCTCT TACCTGCACC TACACCTCCA
20  901  CCTACACCAC CAGCAGTCAC TCCTTTGTTA GGATATGGAG GCGCCATCTT
951  CTGTACTCCT CCAGCTACCC CCCCACCAAC AGGTGTTAGC CTGACTATAT
1001  CTGGAGAAAA CAGCGTTACA TTCCTAGAAA ACATTGCCTC CGAACAAGGA
1051  GGAGCCCTCT ATGGCAAAAA GATCTCTATA GATTCTAATA AATCTACAAT
1101  ATTTCTTGGA AATACAGCTG GAAAAGGAGG CGCTATTGCT ATTCCCGAAT
25  1151  CTGGGGAGCT CTCTCTATCC GCAAATCAAG GTGATATCCT CTTTAACAAG
1201  AACCTCAGCA TCACTAGTGG GACACCTACT CGCAATAGTA TTCACTTCGG
1251  AAAAGATGCC AAGTTTGCCA CTCTAGGAGC TACGCAAGGC TATACCCTAT
1301  ACTTCTATGA TCCGATTACA TCTGATGATT TATCTGCTGC ATCCCGAGCC
30  1351  GCTACTGTGG TCGTCAATCC CAAAGCCAGT GCAGATGGTG CGTATTTCAGG
1401  GACTATGTGC TTTTCAGGAG AAACCCTCAC TGCTACCGAA GCAGCAACCC
1451  CTGCAATGTC TACATCTACA TTAACCAAAA AGCTAGAACT TGAAGGCGGT
1501  ACTCTCGCTT TAAGAAACGG TGCTACCTTA AATGTTTATA ACTTCACGCA
1551  AGATGAAAG TCCGTCGTCA TCATGGATGC AGGGACCACA TTAGCAACTA
35  1601  CAAATGGAGC TAATAATACT GACGGTGCTA TCACCTTAAA CAAGCTTGTA
1651  ATCAATCTGG ATTCTTTGGA TGGCACTAAA GCGGCTGTCT TTAATGTGCA
1701  GAGTACCAAT GGAGCTCTCA CTATATCCGG AACTTTAGGA CTTGTGAAAA
1751  ACTCTCAAGA TTGCTGTGAC AACCACGGGA TGTTTAATAA AGATTACAG
1801  CAACTCCGA TTTTAGAACT CAAAGCGACT TCAAATACTG TAACCACTAC
40  1851  GGACTTCAGT CTCGGCACAA ACGGCTATCA GCAATCTCCC TATGGGTATC
1901  AAGGAACCTG GGAGTTTACC ATAGACACGA CAACCCATAC GGTACACAGG
1951  AATTGAAAAA AAACCGGTTA TCTTCTCAT CCGGAGCGTC TTGCTCCCTT
2001  CATTCCTAAT AGCCTATGGG CAAACGTCAT AGATTACGA GCTGTAAGTC
2051  AAGCGTCAGC AGCTGATGGC GAAGATGTCC CTGGGAAGCA ACTGAGCATC
45  2101  ACAGGAATTA CAAATTTCTT CCATGCGAAT CATACCGGTG ATGCACGCAG
2151  CTACCGCCAT ATGGGTGGAG GCTACCTCAT CAATACCTAC ACACGCATCA
2201  CTCCAGATGC TCGTTAAGT CTAGGTTTGT GACAGCTGTT TACAAAATCT
2251  AAGGATTACC TCGTAGGTCA CGGTCACTCT AACGTTTATT TCGCTACAGT
2301  ATACTCTAAC ATCACCAAGT CTCGTGTTGG ATCATCGAGA TTCTTCTCAG
50  2351  GAGGCACCTC TCGAGTTACC TATAGCCGTA GCAATGAGAA AGTAAAGACT
2401  TCATATACAA AATTGCCTAA AGGGCGCTGC TCTTGAGTA ACAATTGCTG
2451  GTTAGGAGAA CTCGAAGGGA ACCTTCCCAT CACTCTCTCT TCTCGCATCT
2501  TAAACCTCAA GCAGATCATT CCCTTTGTAA AAGCTGAAGT TGCTTACGCG
2551  ACTCATGGGG GCATCCAAGA AAATACCCCC GAGGGGAGGA TTTTGGACA
60  2601  CGGTCATCTA CTCACCGTTG CAGTTCCCGT AGGCGTCCGC TTTGGTAAAA
55  2651  ATTCTCATAA TCGACCAGAT TTTTACACTA TAATCGTAGC CTATGCTCCT
2701  GATGTCTATC GTCACAATCC TGATTGCGAT ACGACATTAC CTATTAATGG
2751  AGCTACGTGG ACCTCTATAG GGAATAATCT AACCAGAAGT ACTTTGCTAG
2801  TACAAGCATC CAGCCATACT TCAGTAAATG ATGTTCTAGA GATCTTCGGG
2851  CACTGTGGAT GTGATATTCG CAGAACCCTC CGTCAATATA CTCTAGATAT
2901  AGGAAGCAAA TTACGATTTT AA

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The PSORT algorithm predicts an outer membrane location (0.917).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 2A, and also as a GST-fusion. Both proteins were used to immunise mice, whose sera were used in a Western blot (Figure 2B) and for FACS analysis (Figure 2C).

The cp6736 protein was also identified in the 2D-PAGE experiment (Cpn0453) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6736 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 3

The following *C.pneumoniae* protein (PID 4376751) was expressed <SEQ ID 5; cp6751>:

```

10      1  MRFFCFGMLL PFTFVLANEG LQLPLETYIT LSPBYQAAPQ VGFTHNQND
      51  LAIVGNHNDP ILDYKYYSRN GGALTCKNLL ISENIGNVFF EKNVCPNSGG
      101  AIYAAQNCTI SKNQNYAFTT NLVSDNPTAT AGSLLGGALF AINCSTNNL
      151  GQGTFFVDNLA LNKGGALYTE TNLSIKDNKG PIIIKQNRAL NSDSLGGGIY
      201  SGNSLNIEGN SGAIQITSNS SGSGGGIFST QTLTISSNKK LIEISENSAF
      251  ANNYGSNFNP GGGGLTTTFC TILNNREGVL FNNNQSQSNG GAIHAKSIII
      301  KENGPVYFLN NTATRGGALL NLSAGSGNGS FILSADNGDI IFNNNTASKH
      351  ALNPPYRNAI HSTPNMNLQI GARPGYRVLF YDPIEHELPS SFPILFNFET
      401  GHTGTVLVFSG EHVHQNFIDE MNFFSYLRNT SELRQGVLA V EDGAGLACYK
      451  FFQRGGTLILL GQGAIVTTAG TIPTPSSTPT TVGSTITLNH LAIDLPSILS
      501  FQAQAPKIWI YPTKTGSTYT EDSNPTITIS GTLTLRNSNN EDPYDSL DLS
      551  HSLEKVP LLY IVDVAAQKIN SSQDLSTLN SGEHYGYQGI WSTYWVETTT
      601  ITNPTSL LGA NTKHKLLYAN WSPLGYRPHP ERRGEFITNA LWQSAYTALA
      651  GLHSLSSWDE EKGAASLQG IGLLVHQKDK NGFRGFRSHM TGYSATTEAT
      701  SSQSPNFSLG FAQFFSKAKE HESQNSTSSH HYFSGMCIEI TLFKEWIRLS
      751  VSLAYMFTSE HTHMTYQGLL EGNSQGSFHN HTLAGALSCV FLPOPHGESL
      801  QIYFPITALA IRGNLAAFQE SGDHAREFSL HRPLTDVSLP VGIRASWKNH
      851  HRVPLVWLTE ISYRSTLYRQ DPELHSKLLI SQGTWTTQAT PVTYNALGIK
      901  VKNFMQVFPK VTLSLDYSAD ISSSTLSHYL NVASRMRF*

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A predicted signal peptide is highlighted.

30 The cp6751 nucleotide sequence <SEQ ID 6> is:

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      1  ATGCCTTTT TTTGCTTCGG AATGTTGCTT CCTTTTACTT TTGTATTGGC
      51  TAATGAAGGT CTCCAAC TTC TTTGGAGAC CTATATTACA TTAAGTCCTG
      101  AATATCAAGC AGCCCTCAA GTAGGGTTTA CTCATAACCA AAATCAAGAT
      151  CTCGCAATTG TCGGGAATCA CAATGATTTC ATCTTGGA CT ATAAGTACTA
      201  TCGGTGCAAT GGAGTGCTC TTACCTGTAA GAATCTCTG ATCTCTGAAA
      251  ATATAGGGAA TGTCTCTTT GAGAAGAATG TCTGTCCCAA TTCTGGCGGG
      301  GCAATTTATG CTGCTCAAAA TTGCACGATC TCCAAGAATC AGAATATGTC
      351  ATTTACTACA AACTTGGTCT CTGACAATCC TACAGCCACT GCGGGATCAC
      401  TATTGGGTGG AGCTCTCTTT GCCATAAAT GCTCTATTAC TAATAACCTA
      451  GGACAGGGAA CTTTCGTTGA CAATCTCGCT TTAATAAGG GGGGTGCCCT
      501  CTATACTGAG ACGAACTTAT CTATTAAAGA CAATAAAGGC CCGATCATAA
      551  TCAAGCAGAA TCGGGCACTA AATTGCGACA GTTTAGGAGG AGGGATTTAT
      601  AGTGGGAAT CTCTAAATAT AGAGGGAAT TCTGGAGCTA TACAGATCAC
      651  AAGCAACTCT TCAGGATCTG GGGGAGGCAT ATTTTCTACC CAAACACTCA
      701  CGATCTCCTC GAATAAAAAA CTCATAGAAA TCAGTGAAAA TTCCGCGTTC
      751  GCAATAACT ATGGATCGAA CTTCAATCCA GGAGGAGGAG GTCTTACTAC
      801  CACCTTTTGC ACGATATTGA ACAACCGAGA AGGGGTACTC TTTAACAATA
      851  ACCAAAGCCA GAGCAACGGT GGAGCCATTC ATCGGAAATC TATCATTATC
      901  AAAGAAAATG GTCTGTATA CTTTPTAAAT AACACTGCAA CTCGGGGAGG
      951  GGCTCTCCTC AACTTATCAG CAGGTCTCTG AAACGGAAGC TTCATCTTAT
      1001 CTGCAGATAA TGGAGATATT ATCTTTAACA ATAATACGGC CTCCAAGCAT
      1051 GCCCTCAATC CTCCATACAG AAACGCCATT CACTCGACTC CTAATATGAA
      1101 TCTGCAATAA GGAGCCGTC CCGGTATCG AGTGCTGTTT TATGATCCCA
      1151 TAGAACATGA GCTCCCTTCC TCCTTCCCCA TACTCTTTAA TTTCGAAACC
      1201 GGTACATACG GTACAGTTTT ATTTTCAGGG GAACATGTAC ACCAGAACTT

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1251 TACCGATGAA ATGAATTTCT TTTCTATT T AAGGAACACT TCGGAACACT
 1301 GTCAAGGAGT CCTTGCTGTT GAAGATGGTG CGGGGCTGGC CTGCTATAAG
 1351 TTCTTCCAAC GAGGAGGCAC TCTACTTCTA GGTCAAGGTG CGGTGATCAC
 1401 GACAGCAGGA ACGATTCCCA CACCATCCTC AACACCAACG ACAGTAGGAA
 5 1451 GTACTATAAC TTAAATCAC ATTGCCATTG ACCTTCCTTC TATTCTTTCT
 1501 TTTCAGCTC AGGCTCCAAA AATTGGGATT TACCCACAA AAACAGGATC
 1551 TACCTATACT GAAGATTCCA ACCCGACAAT CACAATCTCA GGAACCTCTCA
 1601 CCTTACGCAA CAGCAACAAC GAAGATCCCT ACGATAGTCT GGATCTCTCG
 1651 CACTCTCTTG AGAAAGTTCC CCTTCTTTAT ATTGTCGATG TCGCTGCACA
 10 1701 AAAAATTAAC TCTTCGCAAC TGGATCTATC CACATTAAAT TCTGGCGAAC
 1751 ACTATGGGTA TCAAGGCATC TGGTCGACCT ATTGGGTAGA AACTACAACA
 1801 ATCACGAACC CTACATCTCT ACTAGGCGCG AATACAAAAC ACAAGCTGCT
 1851 CTATGCAAAC TGGTCTCCTC TAGGCTACCG TCCTCATCCC GAACGTCGAG
 1901 GAGAATTCAT TACGAATGCC TTGTGGCAAT CGGCATATAC GGCTCTTGCA
 15 1951 GGAATCCACT CCCTCTCCTC CTGGGATGAA GAGAAGGGTC ATGCAGCTTC
 2001 CCTACAAGGC ATTTGGTCTTC TGGTTCATCA AAAAGACAAA AACGGTTTTA
 2051 AGGGATTTTC TAGTCATATG ACAGGTATA GTGCTACCAC CGAAGCAACC
 2101 TCTTCTCAAA GTCCGAATTT CTCTTTAGGA TTTGCTCAGT TCTTCTCCAA
 2151 AGCTAAAGAA CATGAATCTC AAAATAGCAC GTCCTCTCAC CACTATTTCT
 20 2201 CTGGAATGTG CATAGAAAAT ACTCTCTTCA AAGAGTGGAT ACGTCTATCT
 2251 GTGTCTCTTG CTTATATGTT TACCTCGGAA CATACCCATA CAATGTATCA
 2301 GGGTCTCCTG GAAGGGAAC CTACGGGATC TTTCCACAAC CATACCTTAG
 2351 CAGGGGCTCT CTCCTGTGTT TTCCTACCTC AACCTCACGG CGAGTCCCTG
 2401 CAGATCTATC CCTTTATTAC TGCCCTAGCC ATCCGAGGAA ATCTTGCTGC
 25 2451 GTTTCAGAA TCTGGAGACC ATGCTCGGGA ATTTTCCCTA CACCGCCCCC
 2501 TAACGGACGT CTCCCTCCCT GTAGGAATCC GCGCTTCTTG GAAGAACCAC
 2551 CACCGAGTTC CCCTAGTCTG GCTCACAGAA ATTTCTATC GCTCTACTCT
 2601 CTATAGGCAA GATCCTGAAC TCCACTCGAA ATTACTGATT AGCCAAGGTA
 2651 CGTGGACGAC GCAGGCCACT CCTGTGACCT ACAATGCTTT AGGGATCAAA
 30 2701 GTGAAAAATA CCATGCAGGT GTTTCCTAAA GTCACTCTCT CCTTAGATTA
 2751 CTCTGCGGAT ATTTCTTCTC CCACGCTGAG TCACTACTTA AACGTGGCGA
 2801 GTAGAATGAG ATTTTAA

The PSORT algorithm predicts an outer membrane location (0.923).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 3A, and also in his-tagged form. The GST-fusion recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 3B) and for FACS analysis (Figure 3C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6751 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 4

The following *C.pneumoniae* protein (PID 4376752) was expressed <SEQ ID 7; cp6752>:

1 MFGMTPAVYS LQTDLSLEKFA LERDEEFRTS FPLLDLSLSTL TGFSPITTFV
 51 GNRHNSQDI VLSNYKSIDN ILLWTSAGG AVSCNNFLLS NVEDHAFFSK
 45 101 NLAIGTGGAI ACQGACTION NRGPLIFFSN RGLNNASTGG ETRGGAIACN
 151 GDFTISQNGQ TFYFVNNSVN NWGGALSTNG HCRIQSNRAP LLFFNNTAPS
 201 GGGALRSNT TISDNTRPIY FKNNCGNNGG AIQTSVTVAI KNNSGSVIFN
 251 NNTALSGSIN SGNGSGGAIY TTNLSIDNP GTILFNNNYC IRDGGAICTQ
 301 FLTIKNSGHV YFTNNQGNWG GALMLLDST CLLFAEQGNI AFQNNNEVFLT
 50 351 TFGRYNAIHC TPNSNLQLGA NKGYTTAFD PIEHQHPTTN PLIFNPANAH
 401 QGTILFSSAY IPEADYENN FISSSKNTSE LRNGVLSIED RAGWQFYKFT
 451 QRGGILKLGH AASIATPANS ETPSTSVGSQ VIINNLAJNL PSILAKGKAP
 501 TLWLSLQSS APFTEDNNPT ITLSGPTLL NEENRDYDYS IDLSEPLQNI
 551 HLLSLSDVTA RHINTDNFHP ESLNATEHYG YQGIWSPYVW ETITTTNNAS
 55 601 IETANTLYRA LYANWTPLGY KVNPEYQDL ATTPLWQSFH TMFSLLSYN
 651 RTGDSIERP FLEIQGIADG LRVHQNSIPG APGFRIQSTG YSLQASSETS

701 LHQKISLGFQ QFFTRTKKIG SSNNVSAHNT VSSLYVELPW FQEFATSTV
 751 LAYGYGDHHL HSLHPSHQEQ AEGTCYSHLT AAAIGCSFPW QQKSYLHLSP
 801 FVQAIARSH QTAFEEIGDN PRKFVSQKPF YNL/TLPLGIQ GKWQSKFHPV
 851 TEWTLLELSYQ FVLYQQNPQI GVTLLASGGS WDILGHNYVR NALGYKVHNVQ
 5 901 TALFRSLDLF LDYQGSVSSS TSTHHLQAGS TLKP*

The cp6752 nucleotide sequence <SEQ ID 8> is:

1 ATGTTCCGGG TGA CTCTCTGC AGTGTATAGT TTACAAACGG ACTCCCTTGA
 51 AAAGTTTGCT TTAGAGAGGG ATGAAGAGTT TCGTACGAGC TTTCCTCTCT
 101 TAGACTCTCT CTCCACTCTT ACAGGATTTT CTCCAATAAC TACGTTTGTT
 151 GGAAATAGAC ATAATTCCTC TCAAGACATT GTACTTTCTA ACTACAAGTC
 201 TATGTGATAAC ATCCTTCTTC TTTGGACATC GGCTGGGGGA GCTGTGTCTT
 251 GTAATAATTT CTTATTATCA AATGTTGAAG ACCATGCCTT CTTAGTAAA
 301 AATCTCGCGA TTGGGACTGG AGGCGCGATT GCTTGCCAGG GAGCTGCAC
 351 AATCAGCAG AATAGAGGAC CCTTATTTT TTTTCAAGT CGAGGTCTTA
 401 ACAATGCGAG TACAGGAGGA GAAACTCGTG GGGGTGCGAT TGCCTGTAA
 451 GGAGACTTCA CGATTCTCTA AAATCAAGGG ACTTTCTACT TTGTCAACAA
 501 TTCCGTCAAC AACTGGGGAG GAGCCCTCTC CACCAATGGA CACTGCCGCA
 551 TCCAAAGCAA CAGGCGACCT CTACTCTTTT TTAACAATAC AGCCCTTAGT
 601 GGAGGGGGTG CGCTTCGTAG TGAATAACA ACGATCTCTG ATAACACGCG
 651 TCCTATTTAT TTTAAGAACA ACTGTGGGAA CAATGGCGGG GCCATTCAAA
 701 CAAGCGTTAC TGTGCGGATA AAAATAACT CCGGTCGGT GATTTTCAAT
 751 AACAAACAG CGTTATCTGG TTCGATAAAT TCAGGAAATG GTTCAGGAGG
 801 GCGGATTTAT ACAACAAACC TATCCATAGA CGATAACCTT GGAATATTTC
 851 TTTTCAATAA TAACTACTGC ATTGCGGATG GCGGAGCTAT CTGTACACAA
 901 TTTTTCACAA TCAAAAATAG TGGCCACGTA TATTTACCA ACAATCAAGG
 951 AAACCTGGGA GGTGCTCTTA TGCTCTTACA GGACAGCACC TGCCTACTCT
 1001 TCGCGGAACA AGGAAATATC GCATTTCAAA ATAATGAGGT TTTCCTCACC
 1051 ACATTTGGTA GATACAACGC CATACATTGT ACACCAATA GCAACTTACA
 1101 ACTTGGAGCT AATAAGGGGT ATACGACTGC TTTTTTTGAT CCTATAGAAC
 1151 ACCAACATCC AACTACAAAT CCTCTAATCT TTAATCCCAA TGCGAACCAT
 1201 CAGGGAACGA TCTTATTTTC TTCAGCTTAT ATCCAGAAAG CTCTGACTA
 1251 CGAAATAAAT TTCATTAGCA GCTCGAAAAA TACCTCTGAA CTTGCGAATG
 1301 GTGTCTCTC TATCGAGGAT CGTGCGGGAT GGCAATTCTA TAAGTTCAT
 1351 CAAAAGGAG GTATCCTTAA ATTAGGGCAT GCGGCGAGTA TTGCAACAAC
 1401 TGCCCAACTCT GAGACTCCAT CAACTAGTGT AGGCTCCAG GTCATCATTA
 1451 ATAACCTTGC GATTAACCTC CCTCGATCT TAGCAAAAGG AAAAGCTCCT
 1501 ACCTTGTGGA TCCGTCTCTT ACAATCTAGT GCTCCTTTCA CAGAGGACAA
 1551 TAACCTTACA ATTACTTTAT CAGGTCCTCT GACTCTTTA AATGAGGAAA
 1601 AACGCGATCC CTACGACAGT ATAGATCTCT CTGAGCCTTT ACAAAACATT
 1651 CATCTCTTTT CTTTATCGGA TGTAACAGCA CGTCATATCA ATACCGATAA
 1701 CTTTCATCCT GAAAGCTTAA ATGCGACTGA GCATTACGGT TATCAAGGCA
 1751 TCTGGTCTCC TTATGGGTA GAGACGATAA CAACAACAA TAACGCTTCT
 1801 ATAGAGACGG CAAACACCTT CTACAGAGCT CTGTATGCCA ATTGGACTCC
 1851 CTTAGGATAT AAGGTCAATC CTGAATACCA AGGAGATCTT GCTACGACTC
 1901 CCCTATGGCA ATCCTTTTCT ACTATGTTCT CTCTATTAA AAGTTATAAT
 1951 CGAACTGGTG ATTCTGATAT CGAGAGGCCT TTCTTAGAAA TTCAAGGGAT
 2001 TGCCGACGGC CTCCTTGTTC ATCAAAATAG CATCCCCGGG GCTCCAGGAT
 2051 TCCGTATCCA ATCTACAGGG TATTCCTTAC AAGCATCCTC CGAACTTCT
 2101 TTACATCAGA AAATCTCCTT AGGTTTTGCA CAGTCTTCA CCCGCACTAA
 2151 AGAAATCGGA TCAAGCAACA ACGTCTCGGC TCACAATACA GTCTCTTCA
 2201 TTTATGTTGA GCTTCCGTGG TTCCAAGAGG CCTTTGCAAC ATCCACAGTG
 2251 TTAGCGTATG GCTATGGGGA CCATCACCTC CACAGCCTAC ATCCCTCACA
 2301 TCAAGAACAG GCAGAAGGGA CGTGTATAG CCATACATTA GCAGCAGCTA
 2351 TCGGTGTTC TTTCCCTTGG CAACAGAAAT CCTATCTTCA CCTCAGCCCG
 2401 TTCTGTCAGG CAATTGCAAT ACGTCTCTAC CAAACAGCGT TCGAAGAGAT
 2451 TGGTGACAAT CCCCAGAAAT TTGTCTCTCA AAAGCCTTTC TATAATCTGA
 2501 CTTACTCTCT AGGAATCCAA GGAAATGGC AGTCAAAAT CCACGTACCT
 2551 ACAGAATGGA CTCTAGAACT TTCTTACCAA CCGGTACTCT ATCAACAAAA
 2601 TCCCAAAATC GGTGTACAGC TACTTGGCAG CGGAGGTTC TGGGATATCC
 2651 TAGGCCATAA CTATGTTTCG AATGCTTTAG GGTACAAAGT CCACAATCAA
 2701 ACTGCGCTCT TCCGTCTCTC CGATCTATTC TTGGATTACC AAGGATCGGT
 2751 CTCCTCTCTG ACATCTACGC ACCATCTCCA AGCAGGAAGT ACCTTAAAT
 2801 TCTAA

The PSORT algorithm predicts a cytoplasmic location (0.138).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 4A, and also as a GST-fusion. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (4B) and the his-tagged protein was used for FACS analysis (4C).

The cp6752 protein was also identified in the 2D-PAGE experiment (Cpn0467).

- 5 These experiments show that cp6752 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 5

The following *C.pneumoniae* protein (PID 4376850) was expressed <SEQ ID 9; cp6850>:

10 1 MKKAVLIAAM FCGVVSLSSC CRIVDCCFED PCAPSSCNPC EVIRKKERS
51 GGNACGSYVP SCSNFCGSTC CNSQSPQVKG CTSPDGRCKQ *

A predicted signal peptide is highlighted.

The cp6850 nucleotide sequence <SEQ ID 10> is:

15 1 ATGAAGAAAG CTGTTTAAAT TGCTGCAATG TTTTGTGGAG TAGTTAGCTT
51 AAGTAGCTGC TGCCGCATTC TAGATTGTTG TTTTGAGGAT CCTTGCGCAC
101 CCTCTTCTTG CAATCCTTGT GAAGTAATAA GAAAAAAGA AAGATCTTGC
151 GGCGGTAATG CTTGTGGGTC CTACGTTCTT TCTTGTCTTA ATCCATGTPG
201 TTCAACAGAG TGTAACTCTC AAAGCCACA AGTTAAAGGT TGTACATCAC
251 CTGATGGCAG ATGCAAACAG TAA

The PSORT algorithm predicts an inner membrane location (0.329).

- 20 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 5A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 5B) and for FACS analysis (Figure 5B). A his-tagged protein was also expressed.

These experiments show that cp6850 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

25 Example 6

The following *C.pneumoniae* protein (PID 4376900) was expressed <SEQ ID 11; cp6900>:

30 1 MKIKFSWKVN FLICLLAVGL IFFGCSRVKR EVLVGRDATW FPKQFGIYTS
51 DTNAFLNDLV SEINYKENLN INIVNQDWVH LFENLDDKRT QGAFTSVLPT
101 LEMLEHYQFS DPILLTGTVL VVAQDSPYQS IEDLGRLIG VYKFDSSVLV
151 AQNIPDAVIS LYQHVPIALE ALTSNCYDAL LAPVIEVTAL IETAYKGRLE
201 IISKPLNADG LRLAILKGTN GDLLEGFNAG LVKTRRSKY DAIRQRYRLP

The cp6900 nucleotide sequence <SEQ ID 12> is:

35 1 GTGAAGATAA AATTTTCTTG GAAGGTAAAT TTTTAAATAT GTTACTGGC
51 TGTGGGACTG ATCTTTTTCG GGTGCTCTCG AGTAAAAAGA GAAGTTCTCG
101 TAGGTCGTGA TGCCACCTGG TTTCAAAAC AATTCGGCAT TTATACATCC
151 GATACCAACG CATTTTAAAC CGATCTTGTT TCTGAGATTA ACTATAAAGA
201 GAATCTAAAT ATTAATATTC TAAATCAAGA TTGGGTGCAT CTCCTTGAGA
251 ATTTAGATGA TAAAAAGACC CAAGGAGCAT TTACATCTGT ATTGCCTACT
301 CTTGAGATGC TCGAACACTA TCAATTTTCT GATCCCATTT TACTCACAGG
40 351 TCTGTCTCTT TCGTTCGCTC AAGACTCTCC TTACCAATCT ATAGAGGATC
401 TTAAGGTCG TCTTATTGGA GTGTATAAGT TTGACTCTTC AGTCTTGTA
451 GCTCAAAATA TCCCTGACGC TGTGATTAGC CTCTACCAAC ATGTTCCAAT
501 AGCATTGGAA GCCTTAACAT CGAATTGTTA CGACGCTCTT CTAGCTCCTG
551 TAATTGAAGT GACCGCGCTA ATAGAAACAG CATATAAAGG AAGACTGAAA
45 601 ATTTATTTCAA AACCTTAAA CGCAGATGGT TTGCGGCTTG CAATACTGAA

651 AGGGACAAAC GGAGATTTCG TTGAAGGGTT TAACGCAGGA CTTGTGAAAA
 701 CACCACGCTC AGGAAAATAC GATGCTATAA AACAGCGGTA TCGTCTTCCC
 751 TAA

The PSORT algorithm predicts an inner membrane location (0.452).

- 5 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 6A. The recombinant protein was used to immunise mice, whose sera were used for FACS analysis (Figure 6B). A his-tagged protein was also expressed.

The cp6900 protein was also identified in the 2D-PAGE experiment (Cpn0604).

- 10 These experiments show that cp6900 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 7

The following *C.pneumoniae* protein (PID 4377033) was expressed <SEQ ID 13; cp7033>:

1 MVNPIGPGPI DETERTPPAD LSAQGLEASA ANKSAEAQRI AGAEAKPKES
 51 KTDSEVERWSI LRSVNALMS LADKLGIASS NSSSTSRSA DVDSTTATAP
 101 TPPPPTFFDY KTQAQTAYDT IFTSTSLADI QAALVSLQDA VTNIKDTAAT
 151 DEETAIAAEW ETKNADAVKV GAQITELAKY ASDNQAILDS LGKLTFSFDLL
 201 QAALLQSVAN NNKAAELLKE MQDNPVVPKG TPAIAQSLVD QTDATATQIE
 251 KDGNAIRDAY FAGQNASGAV ENAKSNNSIS NIDSAKAAIA TAKTQIAEAQ
 301 KKFDPSPILQ RAEQMVIQAE KDLKNIKPAD GSDVPNPGTT VGGSKQQGSS
 20 351 IGSIRVSMIL DDAENETASI LMSGFRQMIH MFNTENPDSQ AAQQLAAQA
 401 RAAKAAGDDS AAAALADAQK ALEAALGKAG QQQGILNALG QIASAAVSA
 451 GVPPAAASSI GSSVKQLYKT SKSTGSDYKT QISAGYDAYK SINDAYGRAR
 501 NDATRDVINN VSTPALTRSV PRARTEARGP EKTDQALARV ISGNSRTLGD
 551 VYSQVSALQS VMQIIQSNPQ ANNEEIROKL TSAVTKPPQF GYPYVQLSND
 25 601 STQKFIKLE SLFAEGSRTA AEIKALSFET NSLFIQQVLV NIGSLYSGYL
 651 Q*

The cp7033 nucleotide sequence <SEQ ID 14> is:

1 ATGGTTAATC CTATTGGTCC AGGTCCTATA GACGAAACAG AACGCACACC
 51 TCCCGCAGAT CTTCTGCTC AAGGATTGGA GGCGAGTGCA GCAAATAAGA
 30 101 GTGCGGAAGC TCAAAGAATA GCAGGTGCGG AAGCTAAGCC TAAAGAATCT
 151 AAGACCGATT CTGTAGAGCG ATGGAGCATC TTGCGTTCTG CAGTGAATGC
 201 TCTCATGAGT CTGGCAGATA AGCTGGGTAT TGCTTCTAGT AACAGCTCGT
 251 CTCTACTAG CAGATCTGCA GACGTGGACT CAACGACAGC GACCGCACCT
 301 ACGCTCCTC CACCCACGTT TGATGATTAT AAGACTCAAG CGCAAACAGC
 35 351 TTACGATACT ATCTTTACCT CAACATCACT AGCTGACATA CAGGCTGCTT
 401 TGGTGAGCCT CCAGGATGCT GTCCTAATA TAAAGGATAC AGCGCTACT
 451 GATGAGGAAA CCGCAATCGC TGCGGAGTGG GAAACTAAGA ATGCCGATGC
 501 AGTTAAAGTT GGCGCGCAAA TTACAGAATT AGCGAAATAT GCTTCGGATA
 551 ACCAAGCGAT TCTTGACTCT TTAGGTAAAC TGACTTCCTT CGACCTCTTA
 40 601 CAGGCTGCTC TTCTCCAATC TGTCAGAAAC AATAACAAAG CAGCTGAGCT
 651 TCTTAAAGAG ATGCAAGATA ACCCAGTAGT CCCAGGGAAA ACGCTGCAA
 701 TTGCTCAATC TTTAGTTGAT CAGACAGATG CTACAGCGAC ACAGATAGAG
 751 AAAGATGGAA ATGCGATTAG GGATGCATAT TTTGCAGGAC AGAACGCTAG
 801 TGGAGCTGTA GAAATGCTA AATCTAATAA CAGTATAAGC AACATAGATT
 45 851 CAGCTAAAGC AGCAATCGCT ACTGCTAAGA CACAAATAGC TGAAGCTCAG
 901 AAAAAGTTCC CCGACTCTCC AATCTTTCAA GAAGCGGAAC AAATGGTAAT
 951 AAGAGCTGAG AAAGATCTTA AAAATATCAA ACCTGCAGAT GTTCTGATG
 1001 TTCCAAATCC AGGAATACA GTTGGAGGCT CCAAGCAACA AGGAAGTAGT
 1051 ATTGGTAGTA TTCGTGTTTC CATGCTGTTA GATGATGCTG AAAATGAGAC
 50 1101 CGCTTCCATT TTGATGCTCG GTTTCGTCA GATGATTCAC ATGTTCAATA
 1151 CGGAAAATCC TGATTCTCAA GCTGCCAAC AGGAGCTCGC AGCACAAGCT
 1201 AAGAGCGCA AAGCCGCTGG AGATGACAGT GCTGCTGCAG CGCTGGCAGA
 1251 TGCTCAGAAA GCTTTAGAAG CGGCTCTAGG TAAAGCTGGG CAACAACAGG
 1301 GCATACTCAA TGCTTTAGGA CAGATCGCTT CTGCTGCTGT TGTGAGCGCA
 55 1351 GGAGTTCCTC CCGCTGCAGC AAGTTCCTATA GGGTCATCTG TAAACAGCT
 1401 TTACAAGACC TCAAATCTA CAGGTTCTGA TTATAAACA CAGATATCAG

1451 CAGGTTATGA TGCTTACAAA TCCATCAATG ATGCCTATGG TAGGGCACGA
 1501 AATGATGCGA CTCGTGATGT GATAAACAAAT GTAAGTACCC CCGCTCTCAC
 1551 ACGATCCGTT CCTAGAGCAC GAACAGAAGC TCGAGGACCA GAAAAAACAG
 1601 ATCAAGCCCT CGCTAGGGTG ATTTCTGGCA ATAGCAGAAC TCTTGGAGAT
 5 1651 GTCTATAGTC AAGTFTCGGC ACTACAATCT GTAATGCAGA TCATCCAGTC
 1701 GAATCCTCAA GCGAATAATG AGGAGATCAG ACAAAGCTT ACATCGGCAG
 1751 TGACAAAGCC TCCACAGTTT GGCTATCCTT ATGTGCAACT TTCTAATGAC
 1801 TCTACACAGA AGTTCATAGC TAAATAGAA AGTTTGTGTTG CTGAAGGATC
 1851 TAGGACAGCA GCTGAAATAA AAGCACTTTC CTTTGAAACG AACTCCTTGT
 10 1901 TTATTCAGCA GGTGCTGGTC AATATCGGCT CTCTATATTC TGGTTATCTC
 1951 CAATAA

The PSORT algorithm predicts a cytoplasmic location (0.272).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 7A. A
 his-tagged protein was also expressed. The recombinant proteins were used to immunise mice, whose
 15 sera were used for FACS (Figure 7B) and Western blot (7C) analyses.

The cp7033 protein was also identified in the 2D-PAGE experiment (Cpn0728) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7033 a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

20 Example 8

The following *C.pneumoniae* protein (PID 6172321) was expressed <SEQ ID 15; cp0017>:

1 MGIKGTGIIV WVDDATAKTK NATLITWTKTG YKPNPERQGP LVPNSLWGSF
 51 VDVRSIQSLM DRSTSSLSSS TNLWVSGIAD FLHEDQKGNQ RSYRHSSAGY
 101 ALGGGFFTAS ENFFNFAPFCQ LFGYDKDHLV AKNHHTVYAG AMSYRHLGES
 25 151 KTLAKILSGN SDSLPFVFNA RFAYGHTDNN MTKYTGYSY VKGSWGNDAF
 201 GIECGGAIPV VASGRRSWVD THTPFLNLEM IYAHQNDPKE NGTEGRSFQS
 251 EDLFLNLAIPV GIKFEKFSK STYDLNLAIV PDVIRNDPGC TTTLMVSGDS
 301 WSTCGTSLSR QALLVRAGNH HAFASNFVVF SQFEVELRGS SRSYAIDLGG
 351 RFGF*

30 The cp0017 nucleotide sequence <SEQ ID 16> is:

1 ATGGGTATCA AGGGAACCTG AATAATTGTT TGGGTCGACG ATGCAACTGC
 51 AAAAAACAAAA AATGCTACCT TAACCTGGAC TAAAACAGGA TACAAGCCGA
 101 ATCCAGAACG TCAGGGACCT TTGGTTCCTA ATAGCCTGTG GGGTTCCTTT
 35 151 GTCGATGTCC GCTCCATTCA GAGCCTCATG GACCGGAGCA CAAGTTCGTT
 201 ATCTTCGTCA ACAAATTGTT GGGTATCAGG AATCGCGGAC TTTTTCGATG
 251 AAGATCAGAA AGGAAACCAA CGTAGTTATC GTCATTCTAG CGCGGGTTAT
 301 CATTAGGAG GAGGATCTCT CACGGCTTCT GAAAATTCTT TTAATTTTGC
 351 TTTTTCGTCAG CTTTTCGGCT ACGACAAGGA CCATCTTGTG GCTAAGAACCC
 401 ATACCCATGT ATATGCAGGG GCAATGAGTT ACCGACACCT CGGAGAGTCT
 40 451 AAGACCCTCG CTAAGATTTT GTCAGGAAAT TCTGACTCCC TACCTTTTGT
 501 CTTCAATGCT CGGTTTGCTT ATGGCCATAC CGACAATAAC ATGACCACAA
 551 AGTACACTGG CTATCTCTCT GTTAAGGGAA GCTGGGGAAA TGATGCCTTC
 601 GGTATAGAAT GTGGAGGAGC TATCCCGGTA GTTGCTTCAG GACGTCGGTC
 651 TTGGGTGGAT ACCCACACGC CATTTCCTAAA CCTAGAGATG ATCTATGCAC
 45 701 ATCAGATGA CTTTAAGGAA AACGGCACAG AAGGCCGTTT TTCCAAAGT
 751 GAAGACCTCT TCAATCTAGC GGTTCCTGTA GGGATAAAAT TTGAGAAATT
 801 CTCCGATAAG TCTACGTATG ATCTCTCCAT AGCTTACGTT CCCGATGTGA
 851 TTCGTAATGA TCCAGGCTGC ACGACAATC TTATGGTTTC TGGGGATTCT
 901 TGGTCGACAT GTGGTACAAG CTTGTCTAGA CAAGCTCTTC TTGTACGTGC
 50 951 TGGAAATCAT CATGCTTTTG CTTCAAACCT TGAAGTTTTC AGTCAGTTTG
 1001 AAGTCGAGTT GCGAGGTTCT TCTCGTAGCT ATGCTATCGA TCTTGGAGGA
 1051 AGATTCGGAT TTAA

This sequence is frame-shifted with respect to cp0016.

The PSORT algorithm predicts a cytoplasmic location (0.075).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 8A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 8B) and for FACS analysis (Figure 8C). A his-tagged protein was also expressed.

- 5 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp0017 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 9

- 10 The following *C.pneumoniae* protein (PID 6172315) was expressed <SEQ ID 17; cp0014>:

```

1  MKSSPFRFVF STFAIFPLSM IATETVLDSS ASFDGNKNGN FSVRESQEDA
51  GTTYLFGKGV TLENIPGTGT AITKSCFNNT KGDLTFTGNG NSLLFQTVDA
101 GTVAGAAVNS SVVDKSTTFI GFSSLSFIAS PGSSITTGKG AVSCSTGSLs
151 LTKMSVCSSA KTFQRIMAVL SPQKLFH*
```

- 15 The cp0014 nucleotide sequence <SEQ ID 18> is:

```

1  ATGAAGTCIT CTTCCTCCAA GTTTGTATTT TCTACATTG CTATTTTCCC
51  TTTGTCTATG ATTGCTACCG AGACAGTTT GGATTCAAGT GCGAGTTTCG
101 ATGGGAATAA AAATGGTAAT TTTTCAGTTC GTGAGAGTCA GGAAGATGCT
20  GGAAGTACCT ACCTATTTAA GGGAAATGTC ACTCTAGAAA ATATTCCTGG
201 AACAGGCACA GCAATCACAA AAAGCTGTTT TAACAACACT AAGGGCGATT
251 TGACTTTTCA AGGTAAACGGG AACTCTCTAT TGTTCCAAAC GGTGGATGCA
301 GGGACTGTAG CAGGGGCTGC TGTTAACAGC AGCGTGGTAG ATAAATCTAC
351 CACGTTTATA GGGTTTCTTT CGCTATCTTT TATTGCGTCT CCTGGAAGTT
25  CGATAACTAC CGGCAAAGGA GCCGTTAGCT GCTCTACGGG TAGCTTGAGT
451 TTGACAAAAA TGTCAGTTTG CTCCTCAGCA AAAACTTTTC AACGGATAAT
501 GCGGGTGCTA TCACCGCAAA AACTCTTTCA TTAA
```

This protein is frame-shifted with respect to cp0015.

The PSORT algorithm predicts an inner membrane location (0.047).

- 30 The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 9A. A GST-fusion was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in an immunoassay (Figure 9B) and for FACS analysis (Figure 9C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

- 35 These experiments suggest that cp0014 is a useful immunogen. These properties are not evident from the sequence alone.

Example 10

The following *C.pneumoniae* protein (PID 6172317) was expressed <SEQ ID 19; cp0015>:

```

1  MSALFSENTS SKKGAIQTS DALTTTGNQG EVSFDNTSS DSGAAIFTEA
40 51  SVTISNNAKV SFIDNKVTGA SSSTTGDMSS GAICAYKTST DTKVTLTGNQ
101 MLLFSNNTST TAGGAIYVKK LELASGGLTL FSRNSVNGGT APKGGAIIE
151 DSGELSLSD SGDIVFLGNT VTSTTPGTNR SSIDLGTSK MTALRSAAGR
```


-50-

201 AIIFYDPIIT GSSTTVTDVL KVNETPADSA LQYTGNIIFT GEKLSETEAA
 251 DSKNLTSKLL QPVTLSGGTL SLKHGVTLLQT QAFYQQADSR LEMDVGTTLLE
 301 PADTSTINNL VINISSIDGA KKAKIETKAT SKNLTLSGTI TLLDPTGTFY
 5 351 ENHSLRN PQS YDILELKASG TVTSTAVTPD PIMGEKFHYG YQGTWGPVW
 401 GTGASTTATF NWTKTGYIPN PERIGSLVPN SLWNAFIDIS SLHYLMETAN
 451 EGLQGDRAFW CAGLSNFFHK DSTKTTRGFR HLSGGYVIGG NLHTCSDKIL
 501 SAAFCQLFGR DRDYFVAKNQ GTVYGGTLYY QHNETYISLP CKLRPCSLSY
 551 VPTEIPVLFS GNLSYTHTDN DLKTKYTTYP TVKGSWGNDS FALBFGGRAP
 601 ICLDESALFE QYMPFMKLQF VYAHQEGFKE QGTEAREFGS SRLVNLALPI
 10 651 GIRFDKESDC QDATYNLTG YTVDLVRSNP DCTTTLRISG DSWKTFGTNL
 701 ARQALVLRAG NHFCFNSNFE AFSQFSFELR GSSRNYNVDL GAKYQF*

This sequence is frame-shifted with respect to cp0014.

The cp0015 nucleotide sequence <SEQ ID 20> is:

1 ATGTCAGCTC TGTTTTCTGA AAATACCTCC TCAAAGAAAG GCGGAGCCAT
 15 51 TCAGACTTCC GATGCCCTTA CCATTACTGG AAACCAAGGG GAAGTCTCTT
 101 TTTCTGACAA TACTTCTTCG GATTCTGGAG CTGCAATTTT TACAGAAGCC
 151 TCGGTGACTA TTTCTAATAA TGCTAAAGTT TCCTTTATTG ACAATAAGGT
 201 CACAGAGCGC AGCTCCTCAA CAACGGGGGA TATGTCAGGA GGTGCTATCT
 251 GTGCTTATAA AACTAGTACA GATACTAAGG TCACCCTCAC TGGAAATCAG
 20 301 ATGTTACTCT TCAGCAACAA TACATCGACA ACAGCGGGAG GAGCTATCTA
 351 TGTGAAAAAG CTCGAACTGG CTTCCGGAGG ACTTACCCTA TTCAGTAGAA
 401 ATAGTGTCAA TGGAGGTACA GCTCCTAAAG GTGGAGCCAT AGCTATCGAA
 451 AGATGTGGGG AATTGAGTTT ATCCGCCGAT AGTGGTGACA TTGCTTTTTT
 501 AGGGAATACA GTCACCTCTA CTACTCCTGG GACGAATAGA AGTAGTATCG
 25 551 ACTTAGGAAC GAGTGCAAAAG ATGACAGCTT TCGCTTCTGC TGCTGGTAGA
 601 GCCATCTACT TCTATGATCC CATAACTACA GGATCATCCA CAACAGTTAC
 651 AGATGTCTTA AAAGTTAATG AGACTCCGGC AGATTCTGCA CTACAATATA
 701 CAGGGAACAT CATCTTCACA GGAGAAAAGT TATCAGAGAC AGAGGCCGCA
 751 GATTCTAAAA ATCTTACTTC GAAGCTACTA CAGCTGTAA CTCCTTCAGG
 30 801 AGGTACTCTA TCTTTAAAC ATGGAGTGAC TCTGCAGACT CAGGCATTCA
 851 CTCACAGGC AGATTCTCGT CTCGAAATGG ACGTAGGAAC TACTCTAGAA
 901 CCTGCTGATA CTAGCACCAT AAACAATTTG GTCATTAACA TCAGTTCTAT
 951 AGACGGTGCA AAGAAGGCAA AAATAGAAAC CAAAGCTACG TCAAAAATC
 1001 TGACTTTATC TGGAAACCATC ACTTTATTGG ACCCGACGGG CACGTTTAT
 35 1051 GAAAATCATA GTTTAAGAAA TCCTCAGTCC TACGACATCT TAGAGCTCAA
 1101 AGCTTCTGGA ACTGTAAACAA GCACCGCAGT GACTCCAGAT CCTATAATGG
 1151 GTGAGAAAT CCATTACGGC TATCAGGGAA CTTGGGGCCC AATTGTTTGG
 1201 GGGACAGGGG CTTCTACGAC TGCAACCTTC AACTGGACTA AAACGGCTA
 1251 TATTCCTAAT CCCGAGCGTA TCGGCTCTTT AGTCCCTAAT AGCTTATGGA
 40 1301 ATGCATTTAT AGATATTAGC TCTCTCCATT ATCTTATGGA GACTGCAAAAC
 1351 GAAGGGTTGC AGGGAGACCG TGCTTTTGG TGTGCTGGAT TATCTAACTT
 1401 CTTCCATAAG GATAGTACAA AAACACGACG CGGGTTTCGC CATTTGAGTG
 1451 CGGGTTATGT CATAGGAGGA AACCTACATA CTTGTTTACA TAAGATTCTT
 45 1501 AGTGTGTCAT TTTGTGAGCT CTTTGGGAAGA GATAGAGACT ACTTTGTAGC
 1551 TAAGAAATCAA GGTACAGTCT ACGGAGGAAC TCTCTATTAC CAGCACAACG
 1601 AAACCTATAT CTCTCTTCTC TGCAAACTAC GGCCTGTGTC GTTGTCTTAT
 1651 GTTCCTACAG AGATTCCTGT TCTCTTTTCA GGAAACCTTA GCTACACCCA
 1701 TACGGATAAC GATCTGAAAA CCAAGTATAC AACATATCCT ACTGTTAAAG
 1751 GAAGCTGGGG GAATGATAGT TTCCGCTTAG AATTCCGGTGG AAGAGCTCCG
 50 1801 ATTTGCTTAG ATGAAAGTGC TCTATTTGAG CAGTACATGC CCTTCATGAA
 1851 ATTGCAAGTTT GTCTATGCAC ATCAGGAAGG TTTTAAAGAA CAGGGAACAG
 1901 AAGCTCGTGA ATTTGGAAGT AGCCGTCTTG TGAATCTTGC CTTACCTATC
 1951 GGGATCCGAT TTGATAAGGA ATCAGACTGC CAAGATGCAA CGTACAATCT
 2001 AACTCTTGGT TATACTGTGG ATCTTGTTCG TAGTAACCCC GACTGTACGA
 55 2051 CAACACTGCG AATTAGCGGT GATTCTTGGG AAACCTTCGG TACGAATTTG
 2101 GCAAGACAAG CTTTAGTCTT TCGTGACGGG AACCAATTTT GCTTTAACTC
 2151 AAATTTTGA GCCTTTAGCC AATTTCTTTT TGAATGCGT GGGTCATCTC
 2201 GCAATTACAA TGTAGACTTA GGAGCAAAAT ACCAATTCTA A

The PSORT algorithm predicts a cytoplasmic location (0.274).

- 60 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 10A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 10B) and for FACS analysis. A his-tagged protein was also expressed.

These experiments show that cp0015 is a useful immunogen. These properties are not evident from the sequence alone.

Example 11

The following *C.pneumoniae* protein (PID 6172325) was expressed <SEQ ID 21; cp0019>:

```

5      1  LQDSQDYSFV  KLSFGAGGTI  ITQDASQKPL  EVAPSRPHYG  YQGHWNVQVI
      51  PGTGTQPSQA  NLEWVRTGYL  PNPERQGSV  PNSLWGSFVD  QRAIQEIMVN
     101  SSQILQERG  VWGAGIANFL  HRDKINEHGY  RHSGVGYLVG  VGTHAFSDAT
     151  INAAFCQLFS  RDKDYVVSKN  HGTSYSGVVF  LEDTLEFRSP  QGFYTDSSSE
     201  ACCNQVVTID  MQLSYSHRNN  DMKTKYTTYF  EAQGSWANDV  FGLEFGATTY
    10  251  YYPNSTFLFD  YYSFPLRLQC  TYAHQEDFKE  TGGEVRHFTS  GDLFNLAVPI
     301  GVKPERFSDC  KRGSYELTLA  YVPDVIRKDP  KSTATLASGA  TWSTHGNNLS
     351  RQGLQLRLGN  HCLINPGIEV  FSHGAIELRG  SSRNYNINLG  GKYRF*

```

This sequence is frame-shifted with respect to cp0018.

The cp0019 nucleotide sequence <SEQ ID 22> is:

```

15      1  TTGCAAGACT  CTCAAGACTA  TAGCTTTGTA  AAGTTATCTC  CAGGAGCGGG
      51  AGGGACTATA  ATTACTCAAG  ATGCTTCTCA  GAAGCCTCTT  GAAGTAGCTC
     101  CTTCTAGACC  ACATTATGGC  TATCAAGGAC  ATTGGAATGT  GCAAGTCATC
     151  CCAGGAACGG  GAACTCAACC  GAGCCAGGCA  AATTTAGAAT  GGGTGCGGAC
     201  AGGATACCTT  CCGAATCCCG  AACGGCAAGG  ATCTTTAGTT  CCCAATAGCC
    20  251  TGTGGGGTTC  TTTTGTGTAT  CAGCGTGCTA  TCCAAGAAAT  CATGGTAAAT
     301  AGTAGCCAAA  TCTTATGTCA  GGAACGGGGA  GTCTGGGGAG  CTGGAATTGC
     351  TAATTTCTTA  CATAGAGATA  AAATTAATGA  GCACGGCTAT  CGCCATAGCG
     401  GTGTCGGTTA  TCTTGTGGGA  GTTGGCACTC  ATGCTTTTTC  TGATGCTACG
     451  ATAAATGCGG  CTTTGTGCCA  GCTCTTCAGT  AGAGATAAAG  ACTACGTAGT
    25  501  ATCCAAAAAT  CATGGAACCT  GCTACTCAGG  GGTGCTATTT  CTTGAGGATA
     551  CCCTAGAGTT  TAGAAGTCCA  CAGGGATTCT  ATACTGATAG  CTCCCTCAGAA
     601  GCTTGCTGTA  ACCAAGTCGT  CACTATAGAT  ATGCAGTTGT  CTTACAGCCA
     651  TAGAAATAAT  GATATGAAAA  CCAAATACAC  GACATATCCA  GAAGCTCAGG
     701  GATCTTGGGC  AAATGATGTT  TTTGGTCTTG  AGTTTGGAGC  GACTACATAC
    30  751  TACTACCCTA  ACAGTACTTT  TTTATTTGAT  TACTACTCTC  CGTTTCTCAG
     801  GCTGCAGTGC  ACCTATGCTC  ACCAGGAAGA  CTTCAAAGAG  ACAGGAGGTG
     851  AGGTTTCGTC  CTTTACTAGC  GGAGATCTTT  TCAATTTAGC  AGTTCTTATT
     901  GCGGTGAAGT  TTGAGAGATT  TTCAGACTGT  AAAAGGGGAT  CTTATGAACT
     951  TACCCTTGCT  TATGTTCTTG  ATGTGATTCT  CAAAGATCCC  AAGAGCACGG
    35  1001  CAACATTGGC  TAGTGGAGCT  ACGTGGAGCA  CCCACGGAAA  CAATCTCTCC
     1051  AGACAAGGAT  TACAACCTGC  TTTAGGGAAC  CACTGTCTCA  TAAATCCTGG
     1101  AATTGAGGTG  TTCAGTCACG  GAGCTATTGA  ATTGCGGGGA  TCCTCTCGTA
     1151  ATTATAACAT  CAATCTCGGG  GGTAAATACC  GATTTTAA

```

The PSORT algorithm predicts a cytoplasmic location (0.189).

40 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 11A. This protein was used to immunise mice, whose sera were used in a Western blot (Figure 11B) and an immunoblot assay (Figure 11C). A his-tagged protein was also expressed.

These experiments show that cp0019 is a useful immunogen. These properties are not evident from the sequence alone.

45 Example 12

The following *C.pneumoniae* protein (PID 4376466) was expressed <SEQ ID 23; cp6466>:

```

50      1  MRKISVGICI  TILLSLSVVL  QGCKESSHSS  TSRGELAINI  RDEPRSLDPR
      51  QVRLLSEISL  VKHYEGLVQ  ENNLSGNIEP  ALAEDYSLSS  DGLTYTFKLR
     101  SAFWSNGDPL  TAEDFIESWK  QVATQEVSGI  YAFALNPIKN  VRKIQEGHLS
     151  IDHFGVHSPN  ESTLVVTLES  PTSHFLKLLA  LPVFPVHKS  QRTLQSKSLP
     201  IASGAFYPKN  IKQKQWIKLS  KNPHYYNQSQ  VETKTITIHF  IPDANTAACL

```

251 FNQGLNMQG PPWGERIPQE TLSNLQSKGH LHSFDVAGTS WLTPNINKFP
 301 LNNMKLREAL ASALDKEALV STIFLGRAKT ADHLLPTNIH SYPEHQKQEM
 351 AQRQAYAKKL FKEALEELQI TAKDLEHLNL IFPVSSASS LLVQLIREQW
 401 KESLGFAIPI VGKEFALLQA DLSSGNFSLA TGGWFADFAD PMAFLTIFAY
 5 451 PSGVPPYAIN HKDFLEILQN IEQEQDHQKR SELVSQASLY LETFHIIEPI
 501 YHDAFQFAMN KKLNLGVSP TGVVDFRYAK EN*

A predicted signal peptide is highlighted.

The cp6466 nucleotide sequence <SEQ ID 24> is:

1 ATGCGCAAGA TATCAGTGGG AATCTGTATC ACCATTCTCC TTAGCCTCTC
 10 51 CGTAGTCCTC CAAGGCTGCA AGGAGTCCAG TCACTCCTCT ACATCTCGGG
 101 GAGAACTCGC TATTAATATA AGAGATGAAC CCCGTTCCTT AGATCCAAGA
 151 CAAGTGCAGC TTCTTTCAGA AATCAGCCTT GTCAAACATA TCTATGAGGG
 201 ATTAGTTCAA GAAATAATC TTTCAGGAAA TATAGAGCCT GCTCTTGCAG
 251 AAGACTACTC TCCTTCCTCG GACGACTCA CTTATACTTT TAAACTGAAA
 15 301 TCAGCTTTT GAGTAATGG CGACCCCTTA ACAGCTGAAG ACTTTATAGA
 351 ATCTTGGAAA CAAGTAGCTA CTCAGGAAGT CTCAGGAATC TATGCTTTTG
 401 CCTTGAATCC AATFAAAAT GTACGAAAGA TCCAAGAGGG ACACCTCTCC
 451 ATAGACCAAT TTGGAGTGCA CTCCTCTAAT GAATCTACAC TTGTGTGTAC
 501 CCTTGAATCC CCACCTCGC ATTTCTTAAA ACTTTTAGCT CTTCAGTCT
 20 551 TTTTCCCCGT TCATAAATCT CAAAGAACCC TGCAATCCAA ATCTCTACCT
 601 ATAGCAAGCG GAGCTTTCTA TCCTAAAAAT ATCAAAACAA AACAATGGAT
 651 AAAACTCTCA AAAAACCTC ACTACTATA TCAAAGTCAG GTGGAACTA
 701 AAACGATTAC GATTCACITC ATTCCCGATG CAAACACAGC AGCAAACTA
 751 TTTAATCAGG GAAAACCTCA TTGGCAAGGA CCTCTTGGG GAGAACGCAT
 25 801 TCCTCAAGAA ACCCTATCCA ATTTACAGTC TAAGGGGCAC TTACTACTCT
 851 TTGATGTGCG AGGAACCTCA TGGCTCACCT TCAATATCAA TAAATTCCTC
 901 CTCACAAATA TGAAGCTTAG AGAAGCCTTA GCATCAGCCT TAGATAAGGA
 951 AGCTCTTGTC TCAACTATAT TCTTAGGCCG TGCAAAACT GCCGATCATC
 1001 TCCTACCTAC AATATTCAT AGCTATCCCG AACATCAAAA ACAAGAGATG
 30 1051 GCACAACGCC AAGCTTACGC TAAAAAATC TTTAAGAAG CTTTAGAAGA
 1101 ACTCAAATC ACTGCTAAAG ATCTCGAACA TCTTAATCTT ATCTTTCCTC
 1151 TTCTCTCGTC AGCAAGTTCT TTAGTAGTCC AACTTATACG AGAACAGTGG
 1201 AAAGAAAGTT TAGGGTTCGC TATCCCTATT GTCGGAAGG AATTGCTCT
 1251 TCTCAAGCA GACCTATCTT CAGGGAACCT CTCCTTAGCT ACAGGAGGAT
 35 1301 GGTTCGAGA CTCTGCTGAT CCTATGGCAT TTCTAACGAT CTTTGTCTAT
 1351 CCATCAGGAG TTCTCTCTTA TGCAATCAAC CATAAGGACT TCCTAGAAAT
 1401 TCTACAAAC ATAGAACAAG AGCAAGATCA CCAAAACGC TCAGGAATTAG
 1451 TGTCGCAAGC TTCTCTTTAC CTAGAGACCT TTCATATTAT TGAGCCGATC
 1501 TACCACGACG CATTTCAAT TGCTATGAAT AAAAACTTT CTAATCTAGG
 40 1551 AGTCTCACCA ACAGGAGTTG TGGACTTCCG TTATGCTAAG GAAAATTAG

The PSORT algorithm predicts that the protein is an outer membrane lipoprotein (0.790).

The protein was expressed in *E.coli* and purified both as a GST-fusion product and a His-tag fusion product. Purification of the protein as a GST-fusion product is shown in Figure 12A. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 12B and 12C). FACS analysis was also performed.

These experiments show that cp6466 is a useful immunogen. These properties are not evident from the sequence alone.

Example 13

The following *C.pneumoniae* protein (PID 4376468) was expressed <SEQ ID 25; cp6468>:

50 1 MFSRWITLFL LFISLTGCS YSSKHQSLI IPIHDDPVAF SPEQAKRAMD
 51 LSIAQLLFDG LTRETHRESN DLRLAIASRY TVSEDFCSYT FFIKDSALWS
 101 DGTPITSEDI RNAWEYAQEN SPHIQIFQGL NFSTPSSNAI TIHLDSPNPD
 151 FPKLLAFPAF AIFKPNPKL FSGPYTLVEY FPGHNIHLKK NPNYYDYHCV
 201 SINSIKLLII PDIYTAIHL NRKVDWVGQ PWHQGI PWEL HKQSQYHYT
 55 251 YPVEGAFWLC LNTKSPHLND LQNRHRLATC IDKRSIIEEA LQGTQQPAET

301 LSRGAPQPNQ YKKQKPLTPQ EKLVLTYPSD ILRCQRIARI LKEQWKAAGI
 351 DLILEGLEHYH LFNKRKRVQD YAIATQTGVA YYPGANLISE EDKLLQNFEI
 401 IPIYLLSYDY LTQDFIEGVI YNAGAVDLK YTYFP*

A predicted signal peptide is highlighted.

5 The cp6468 nucleotide sequence <SEQ ID 26> is:

1 ATGTTTTCAC GATGGATCAC CCTCTTTTTA TTATTCATTA GCCTTACTGG
 51 ATGCTCCTCC TACTCTTCAA AACATAACA ATCTTTAATT ATTCCCATAC
 101 ATGACGACCC TGTAGCTTTT TCTCTGAAC AAGCAAAACG GGCCATGGAC
 151 CTTTCTATTG CCCAACTTCT TTTTGATGGT CTGACTAGAG AAACATCATCG
 10 201 CGAATCCAAT GATTTGGAAT TAGCGATTGC CAGTCGCTAT ACAGTCTCTG
 251 AAGACTTTTG CTCTTATACG TTCCTTATCA AAGACAGCGC TTTATGGAGC
 301 GACGGAACAC CAATCACCTC CGAAGATATC CGTAACGCTT GGGAGTATGC
 351 ACAGGAGAAC TCTCCCCACA TACAGATCTT CCAAGGACTT AACTTCTCAA
 401 CTCTTCATC AAATGCAATT ACGATTATC TCGACTCGCC CAACCCGAT
 15 451 TTTCTTAAGC TTCCTGCTTT TCCTGCATTT GCTATCTTTA AACCAGAAAA
 501 CCCGAAGCTC TTTAGCGGTC CGTATACTCT TGTAGAGTAT TTCCCAGGGC
 551 ATAACATTCA TTTAAAGAAA AACCCTAACT ATTACGACTA CCACGCGTC
 601 TCCATCAACT CCATCAAACT GCTCATTATT CCTGATATAT ATACAGCCAT
 651 CCACCTCCTA AACAGAGGCA AGGTGGACTG GGTAGACAA CCCTGGCATC
 20 701 AAGGGATTCC TTGGGAGCTC CATAACAAT CGCAATATCA CTACTACACC
 751 TATCCTGTAG AAGGTGCCTT CTGGCTTTGT CTAATACAA AATCCCCACA
 801 CTTAAATGAT CTTCAAAACA GACATAGACT CGCTACTTGT ATTGATAAAC
 851 GTTCTATCAT TGAAGAAGCT CTTCAAGGAA CCCAACAACC AGCGGAAACA
 901 CTGTCCCGAG GAGCTCCACA ACCAAATCAA TATAAAAAAC AAAAGCCTCT
 25 951 AACTCCACAA GAAAAACTCG TGCTTACCTA TCCCTCAGAT ATTCTAAGAT
 1001 GCCAACGCAT AGCAGAAATC TTAAGGAAC AATGGAAAGC TGCTGGAATA
 1051 GATTTAATCC TTGAAGGACT CGAATACCAT CTGTTTGTTA ACAAACGAAA
 1101 AGTCCAAGAC TACGCCATAG CAACACAGAC TGGAGTTGCT TATTACCCAG
 1151 GAGCAAATCT AATTTCTGAA GAAGACAAGC TCCTGCAAAA CTTTGAGATT
 30 1201 ATCCCGATCT ACTATCTGAG CTATGACTAT CTCACTCAAG ATTTTATAGA
 1251 GGGAGTAATC TATAATGCTT CTGGAGCTGT AGATCTCAA TATACCTATT
 1301 TCCCCTAG

The PSORT algorithm predicts that this protein is an outer membrane lipoprotein (0.790).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 13A.

35 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 13B) and for FACS analysis. A his-tagged protein was also expressed.

These experiments show that cp6468 is a useful immunogen. These properties are not evident from the sequence alone.

Example 14

40 The following *C.pneumoniae* protein (PID 4376469) was expressed <SEQ ID 27; cp6469>:

1 MKMHLKPTL KSLIPNLLFL LLTLSSCSKQ KQEPLGKHLV IAMSHDLADL
 51 DPRNAYLSRD ASLAKALYEG LTRETDQGIA LALAESYTLS KDHKVYTFKL
 101 RPSVWSDGTP LTAYDFEKSI KQLYFEEFSP SIHTLLGVIK NSSAIHNAQK
 151 SLETGLIQAK DDLTLVITL QPFPYFLTLI ARPVFSFVHH TLRESYKKG
 45 201 PPSTYISNGP FVLKKHEHQN YLILEKNPHY YDHESVKLDR VTLKIIPDAS
 251 TATKLKFSKS IDWIGSPWSA PISNEDQKVL SQEKILTVSV SSTLLIYNL
 301 QKPLIQNKAL RKALAHADR KSILRLVPSG QEAVTLVPPN LSQNLQKEI
 351 STEERQTKAR AYFQEAETL SEKELAELSI LYPIDSSNSS IIAQEIQRQL
 401 KDTLGLKIKI QGMEYHCFLK KRRQGDFFIA TGGWIAEYVS PVAFLSILGN
 50 451 PRDLTQWRNS DYEKTLKLY LPHAYKENLK RAEMIIEET PIIPLYHGKY
 501 IYAIHPKIQN TFGSLGHTD LKNIDILS*

A predicted signal peptide is highlighted.

The cp6469 nucleotide sequence <SEQ ID 28> is:

1 ATGAAGATGC ATAGGCTTAA ACCTACCTTA AAAAGTCTGA TCCCTAATCT
 51 TCTTTTCTTA TTGCTCACTC TTTCAGCTG CTCAAAGCAA AAACAAGAAC
 101 CCTTAGGAAA ACATCTCGTT ATTGCGATGA GCCATGATCT CGCCGACCTA
 151 GATCCCTCGCA ATGCCTATTT AAGCAGAGAT GCTTCCCTAG CAAAAGCCCT
 5 201 CTATGAAGGA CTGACAAGAG AAACGTATCA AGGAATCGCA CTGGCTCTTG
 251 CAGAAAGTTA TACCTGTGCA AAAGATCATA AGGTCTATAC CTTTAAACTC
 301 AGACCTTCTG TGTGGAGCGA TGGCACTCCA CTCCTGCTT ATGACTTTGA
 351 AAAATCTATA AAACAACGTG ACTTCGAAGA ATTTTCACCT TCCATACATA
 401 CTTTACTCGG CGTGATTAATA AATTCTTCGG CAATCCACAA TGCTCAAAAA
 10 451 TCTCTGGAAT CTCTTGGGAT ACAGGCAAAA GATGATCTTA CTTTGGTGAT
 501 TACCCCTAGAG CAACCTTTCC CATACTTTCT CACACTTATC GCTCGCCCCG
 551 TATTCTCCCC TGTTCATCAC ACCCTTAGGG AATCCTATAA GAAAGGAACA
 601 CCCCCTATCCA CATACTCTC CAATGGGCC TTTGTCTTAA AAAACATGA
 651 ACACCAAAAC TACTTAATTT TAGAAAAAAA TCCTCACTAC TATGATCATG
 15 701 AATCAGTAAA GTTAGACCGA GTCACCTTAA AAATTATCCC AGACGCCTCC
 751 ACAGCCACGA AACTTTTCAA AAGTAAATCT ATAGATTGGA TTGGCTCACC
 801 TTGGAGCGCT CGATATCTA ACGAAGACCA AAAAGTCTC TCCCAAGAAA
 851 AGATTCTTAC CTAATCTGTT TCAAGACCA CCCTTCTTAT CTATAACCTG
 901 CAAAAACCTC TAATACAAAA TAAAGCCCTC AGGAAAGCCA TTGCTCATGC
 20 951 TATTGATAGA AAATCTATCT TAAGACTCGT GCCTTCAGGA CAAGAAGCTG
 1001 TAACCTTAGT TCCCCCAAAT CTTTCACAAC TCAATCTTCA AAAAGAGATC
 1051 TCAACAGAAAG AACGACAAAC AAAAGCCAGA GCATATTTTC AAGAAGCTAA
 1101 AGAAACACTT TCTGAAAAAG AACTCGCAGA ACTCAGCATC CTCTATCCTA
 1151 TAGATTCTCT GAATTCCTCC ATCATAGCTC AAGAAATCCA AAGACAACCT
 25 1201 AAAGATACCT TAGGATTGAA AATCAAAATC CAAGGCATGG AGTACCACTG
 1251 CTTTTTAAAG AAACGTCGTC AAGGAGATT TTTTCATAGC ACAGGAGGAT
 1301 GGATTGCGGA ATACGTAAGC CCCGTAGCCT TCCTATCTAT TCTAGGCAAC
 1351 CCCAGAGACC TCACACAATG GAGAAACAGT GATTACGAAA AGACTTTAGA
 1401 GAACTCTAT CTCCCTCATG CCTACAAAGA GAATTTAAAA CGCGCAGAAA
 30 1451 TGATAATAGA AGAAGAAACC CCGATTATCC CCCTGTATCA CGGCAATAT
 1501 ATTTACGCTA TACATCTTAA AATCCAGAAT ACATTGCGAT CTCTTCTAGG
 1551 CCACACAGAT CTCAAAAATA TCGATATCTT AAGTTAG

The PSORT algorithm predicts a periplasmic location (0.934).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 14A.

35 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 14B) and for FACS analysis. A his-tagged protein was also expressed.

These experiments show that cp6469 is a useful immunogen. These properties are not evident from the sequence alone.

Example 15

40 The following *C.pneumoniae* protein (PID 4376602) was expressed <SEQ ID 29; cp6602>:

1 MAASGGTGGL GGTQGVNLAA VEAAAAKADA AEVVASQEGS EMNMIQQSQD
 51 LTNPAATRT KKKEEFQTL ESRKKGEAGK AEKKSESTEE KPDTDLADKY
 101 ASGNSEISGQ ELRGLRDAIG DDASPEDILA LVQEKIKDPA LQSTALDYLV
 151 QTTPPSQGKL KEALIQARNT HTEQFGRTAI GAKNILFASQ EYADQLNVSP
 45 201 SGLRSLYLEV TGDTHTCQDL LSMLQDRYTY QDMAIVSSFL MKGMATELKR
 251 QGPYVPSAQL QVLMTEIRNL QAVLTSYDYF ESRVPILLDS LKAEGIQTPS
 301 DLNFVKVAES YHKIINDKFP TASKVEREVR NLIGDDVDSV TGVNLNFFSA
 351 LRQTSRLFS SADKROQLGA MIANALDAVN INNEDYPKAS DFPKPYPWS*

The cp6602 nucleotide sequence <SEQ ID 30> is:

50 1 ATGGCAGCAT CAGGAGGCAC AGGTGGTTTA GGAGGCACTC AGGGTGTCAA
 51 CCTTGACGCT GTAGAAGCTG CAGCTGCAAA AGCAGATGCA GCAGAAGTTG
 101 TAGCCAGCCA AGAAGGTTCT GAGATGAACA TGATTCAACA ATCTCAGGAC
 151 CTGACAAATC CCGCAGCAGC AACACGCACG AAAAAAAGG AAGAGAAGTT
 201 TCAAACTCTA GAATCTCGGA AAAAAGGAGA AGCTGGAAGG GCTGAGAAAA
 55 251 AATCTGAATC TACAGAAGAG AAGCCTGACA CAGATCTTGC TGATAAGTAT
 301 GCTTCTGGGA ATTCTGAAAT CTCTGGTCAA GAACCTTCGC GCCTGCGTGA
 351 TGCAATAGGA GACGATGCTT CTCCAGAAGA CATTCCTGCT CTTGTACAAG

401 AGAAAATTAA AGACCCAGCT CTGCAATCCA CAGCTTTGGA CTACCTGGTT
 451 CAAACGACTC CACCTTCCCA AGGTAAATTA AAAGAAGCGC TTATCCAAGC
 501 AAGGAATACT CACACGGAGC AATTCGGACG AACTGCTATT GGTGCGAAAA
 551 ACATCTTATT TGCCTCTCAA GAATATGCAG ACCAACTGAA TGTTCCTCCT
 5 TCAGGGCTTC GCTCTTTGTA CTTAGAAGTG ACTGGAGACA CACATACCTG
 601 TGATCAGCTA CTTTCTATGC TTCAAGACCG CTATACCTAC CAAGATATGG
 651 CTATTTGTCAG CTCCTTTCTA ATGAAAGGAA TGGCAACAGA ATTA AAAAGG
 701 CAGGGTCCCT ACGTACCCAG TCGGCAACTA CAAGTTCTCA TGACAGAAAC
 751 TCGTAACCTG CAAGCAGTTC TTACCTCGTA CGATTACTTT GAAAGTCGCG
 801 TTCTATTTTT ACTCGATAGC TTA AAAAGCTG AGGGAATCCA AACTCCTTCT
 851 GATCTAAACT TTGTGAAGGT AGCTGAGTCC TACCATAAAA TCATTAACGA
 901 TAAGTCCCA ACAGCATCTA AAGTAGAACG AGAAGTCCGC AATCTCATAG
 951 GAGACGATGT TGATTCTGTG ACCGGTGTCT TGA ACTTATT CTTTCTGCT
 1001 TTACGTCAA CGTCGTCACG CCTTTTCTCT TCAGCAGACA AACGTCAGCA
 1051 ATTAGGAGCT ATGATTGCTA ATGCTTTAGA TGCTGTAAAT ATAAACAATG
 1101 AAGATTATCC CAAAGCATCA GACTTCCCTA AACCTATCC TTGGTCATGA
 1151

The PSORT algorithm predicts a cytoplasmic location (0.080).

The protein was expressed in *E.coli* and purified as both a His-tag and a GST-fusion product, as shown in Figure 15A. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 15B) and for FACS analysis (Figure 15C).

The cp6602 protein was also identified in the 2D-PAGE experiment (Cpn0324).

These experiments show that cp6602 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 16

25 The following *C.pneumoniae* protein (PID 4376727) was expressed <SEQ ID 31; cp6727>:

1 MKYSLPWLIT SSALVFSLHP LMAANTDLSS SDNYENGSSG SAAFTAKETS
 51 DASGTYTTLT SDVSI TNVSA ITPADKSCFT NTGGALSFVG ADHSLVLQTI
 101 ALTHDGAAIN NTNTALSFSG FSSLLIDSAP ATGTSGGKGA ICVTNTEGGT
 151 ATFTDNASVT LQKNTSEKDG AAVSAYSIDL AKTTTAALLD QNTSTKNGGA
 201 LCSTANTTVQ GNSGTVTFSS NTATDRGGGI YSKEKDSLTD ANTGVVTFKS
 251 NTAKTGGAWS SDDNLALTGN TQVLFQENKT TGSAAQANNP EGCGGAI CCY
 301 LATATDKTGL AISQNQEMSF TSNTTTANGG AIYATKCTLD GNTTLTFDQN
 351 TATAGCGGAI YTETEDFSLK GSTGTVTFST NTAKTGGALY SKGNSSLTGN
 401 TNLLFSGNKA TGPSNSSANQ EGCGGAILAF IDSGSVSDKT GLSIANNQEV
 451 SLTSNAATVS GGAIYATKCT LTGNGSLTFD GNTAGTSGGA IYTETEDFTL
 501 TGSTGTVTF S TNTAKTGGAL YSKGNNSLSG NTNLLFSGNK ATGPSNSSAN
 551 QEGCGGAILS FLESASVSTK KGLWIEDNEN VLSGNTATV SGGAIYATKC
 601 ALHGNTTLTF DGNTAETAGG AIYTETEDFT LTGSTGTVTF STNTAKTAGA
 651 LHKTGNTSFT KNKALVPSGN SATATATTTT DQEGCGGAIL CNISESDIAT
 701 KSLTLTENES LSFINNIAKR SGGGIYAPKC VISGSESINF DGNTAETSGG
 751 AIYSKNLSIT ANGPVSFTNN SGGKGGAIYI ADSGELSLEA IDGDTFSGN
 801 RATEGTSTPN SIHLGAGAKI TKLAAAPGHT IYFYDPITME APASGGTIEE
 851 LVINPVVKAI VPPPQPKNGP IASVPVVPVA PANPNTGTIV FSSGKLPSQD
 901 ASIPANTTTI LNQKINLAGG NVVLKEGATL QVYSFTQOPD STVFMDAGTT
 951 LETTTTNNFTD GSIDLKNSLV NLDALDGKRM ITIAVNSTSG GLKISGDLKF
 1001 HNEGFSFYDN PGLKANLNL PFLDLSSTSGT VNLDDFNPIP SSMAAPDYGY
 1051 QGSWTLVPKV GAGGRVTLVA EWQALGYTPK PELRATLVPN SLWNAYVNIH
 1101 SIQOEIATAM SDAPSHPGIW IGGIGNAFHQ DKQKENAGFR LISRGYIVGG
 1151 SMTTPQEYTF AVAFSQLFGK SKDYVVS DIK SQVYAGSLCA QSSYVILPHS
 1201 SLRRHVLSKV LPELPGETPL VLHGQVSYGR NHHNMTTKLA NNTQGRSDWD
 1251 SHSFAVEVGG SLPVDLNYRY LTSYSPYVKL QVSVNQKGF QEVAADPRIF
 1301 DASHLVN VSI FMGLTFKHES AKPPSALLLT LGYAVDAYRD HPHCLTSLTN
 1351 GTSWSTFATN LSRQAFPAEA SGHLKLLHGL DCFASGSCEL RSSRSRYNAN
 1401 CGTRYSF*

55 A predicted signal peptide is highlighted.

The cp6727 nucleotide sequence <SEQ ID 32> is:

```

1  ATGAAATATT CTTTACCTTG GCTACTTACC TCTTCGGCTT TAGTTTTCCTC
51 CCTACATCCA CTAATGGCTG CTAACACGGA TCTCTCATCA TCCGATAACT
101 ATGAAAATGG TAGTAGTGGT AGCGCAGCAT TCACTGCCAA GGAAACTTCG
5  GATGCTTCAG GAACTACCTA CACTCTCACT AGCGATGTTT CTATTACGAA
201 TGTATCTGCA ATTACTCCTG CAGATAAAAG CTGTTTACCA AACACAGGAG
251 GAGCATTGAG TTTTGTGTGA GCTGATCACT CATTGGTTCT GCAAACCATA
301 GCGCTTACGC ATGATGGTGC TGCAATTAAC AATACCAACA CAGCTCTTTC
351 TTTCTCAGGA TTCTCGTCAC TCTTAATCGA CTCAGCTCCA GCAACAGGAA
10  CTTCGGGCGG CAAGGGTGCT ATTTGTGTGA CAAATACAGA GGGAGTACT
451 GCGACTTTTA CTGACAATGC CAGTGTCACT CTCCAAAAAA ATACTTCAGA
501 AAAAGATGGA GCTGCAGTTT CTGCCACAG CATCGATCTT GCTAAGACTA
551 CGACAGCAGC TCTCTTAGAT CAAAATACTA GCACAAAAAA TGGCGGGGCC
601 CTCTGTAGTA CAGCAAAACAC TACAGTCCAA GGAAACTCAG GAACGGTGAC
15  CTTCCTCTCA AATACTGCTA CAGATAAAGG TGGGGGGATC TACTCAAAAG
701 AAAAGGATAG CACGCTAGAT GCCAATACAG GAGTCGTTAC CTTCAAATCT
751 AATACTGCAA AGACGGGGGG TGCTTGGAGC TCTGATGACA ATCTTGCTCT
801 TACCGGCAAC ACTCAAGTAC TTTTTCAGGA AAATAAAACA ACCGGCTCAG
851 CAGCACAGGC AAATAACCCG GAAGGTGTG GTGGGGCAAT CTGTTGTTAT
20  CTTGCTACAG CAACAGACAA AACTGGATTA GCCATTTCTC AGAATCAAGA
901 AATGAGCTTC ACTAGTAATA CAACAACCTG GAATGGTGA GCGATCTACG
1001 CTACTTAAATG TACTCTGGAT GGAAACACAA CTCTTACCTT CGATCAGAAT
1051 ACTGCGACAG CAGGATGTGG CGGAGCTATC TATACAGAAA CTGAAGATTT
1101 TTCTCTTAAG GGAAGTACGG GAACCGTGAC CTTCAGCACA AATACAGCAA
25  AGACAGGCGG CGCCTTATAT TCTAAAGGAA ACAGCTCGCT GACTGGAAAT
1201 ACCAACCTGC TCTTTTCAGG GAACAAAGCT ACGGGCCCGA GTAATTCTTC
1251 AGCAAATCAA GAGGGTGTGG GTGGGGCAAT CCTAGCCTTT ATTGATTCAG
1301 GATCCGTAAG CGATAAAACA GGACTATCGA TTGCAAAACA CCAAGAAGTC
1351 AGCCTCACTA GTAAATGTGC AACAGTAAGT GGTGGTGCAG TCTATGCTAC
30  CAAATGTACT CTAATGGAA ACGGCTCCCT GACCTTTGAC GGCAACTACTG
1401 CTGGAACCTC AGGAGGGGCG ATCTATACAG AAACGGAAGA TTTTACTCTT
1501 ACAGGAAGTA CAGGAACCGT GACCTTCAGC ACAAATACAG CAAAGACAGG
1551 CGGCCTCTTA TATTCTAAAG GCAACAACCT TCTGTCTGGT AATACCAACC
1601 TGCTCTTTTC AGGGAACAAA GCTACGGGCC CGAGTAATTC TTCAGCAAT
35  CAAGAGGGTT GCGGTGGGGC AATCCTATCG TTTCTTGAGT CAGCATCTGT
1701 AAGTACTAAA AAAGGACTCT GGATTGAAGA TAACGAAAAC GTGAGTCTCT
1751 CTGTAAATAC TGCAACAGTA AGTGGCGGTG CGATCTATGC GACCAAGTGT
1801 GCTCTGCATG GAAACACGAC TCTTACCTTT GATGGCAATA CTGCCGAAAC
40  TGCAGGAGGA GCGATCTATA CAGAAACCGA AGATTTTACT CTTACGGGAA
1901 GTACGGGAAC CGTGACCTTC AGCACAAATA CAGCAAAGAC AGCAGGGGCT
1951 CTACATACTA AAGGAAATAC TTCTTTTACC AAAAAAAGG CTCTTGTTAT
2001 TTCTGGAAAT TCAGCAACAG CAACAGCAAC AACAACCTACA GATCAAGAAG
2051 TTGTGTGGTG AGCGATCTCT TGTAATATCT CAGAGTCTGA CATAGCTACA
2101 AAAAGCTTAA CTCTTACTGA AAATGAGAGT TTAAGTTTCA TTAACAATAC
45  GGCAAAAAGA AGTGGTGGTG GTATTTATGC TCCTAAGTGT GTAATCTCAG
2201 GCAGTGAATC CATAAACTTT GATGGCAATA CTGCTGAAAC TTCGGGAGGA
2251 GCGATTTATT CGAAAAACCT TTCGATTACA GCTAACGGTC CTGCTCTCCT
2301 TACCAATAAT TCTGGAGGCA AGGGAGGCGC CATTTATATA GCCGATAGCG
2351 GAGAACTTTC CTTAGAGGCT ATTGATGGGG ATATTACTTT CTCAGGGAAC
50  CGAGCGACTG AGGGAACCTT AACTCCCAAC TCGATCCATT TAGGTGCAGG
2451 GGCTAAGATC ACTAAGCTTG CAGCAGCTCC TGGTCATACG ATTTATTTTT
2501 ATGATCCTAT TACGATGGAA GCTCCTGCAT CTGGAGGAAC AATAGAGGAG
2551 TTAGTCATCA ATCCTGTGTG CAAAGCTATT GTTCTCTCTC CCCAACCAAA
2601 AAATGGTCCT ATAGCTTCAG TGCCTGTAGT CCCTGTAGCA CCTGCAAACC
55  CAAACACGGG AACTATAGTA TTTTCTTCTG GAAAACCTCC CAGTCAAGAT
2701 GCCTCGATTG CTGCAAAATC TACCACCATA CTGAACCAGA AGATCAACTT
2751 AGCAGGAGGA AATGTCGTTT TAAAAGAAGG AGCCACCTTA CAAGTATATT
2801 CCTTCACACA GCAGCCTGAT TCTACAGTAT TCATGGATGC AGGAACGACC
2851 TTAGAGACCA CGACAACATA CAATACAGAT GGCAGCATCG ATCTAAAGAA
60  TCTCTCTGTA AATCTGGATG CTTTAGATGG CAAGCGTATG ATAACGATG
2951 CCGTAAACAG CACAAGTGGG GGATTAAAAA TCTCAGGGGA TCTGAAATTC
3001 CATAACAATG AAGGAAGTTT CTATGACAAT CCTGGGTGTA AAGCAAACCT
3051 AAATCTTCCT TTCTTAGATC TTTCTTCTAC TTCAGGAACT GTAAATTTAG
3101 ACGACTTCAA TCCGATTCCT TCTAGCATGG CTGCTCCGGA TTATGGGTAT
65  CAAAGGAGTT GGAAGCTGTT TCCTAAAGTA GGAGCTGGAG GGAAGGTGAC
3201 TTTGGTTCGG GAATGGCAAG CGTTAGGATA CACTCTTAAA CCAGAGCTTC
3251 GTGCGACTTT AGTTCCTAAT AGCCTTTGGA ATGCTTATGT AAACATCCAT

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-57-

3301 TCTATACAGC AGGAGATCGC CACTGCGATG TCGGACGCTC CCTCACATCC
 3351 AGGGATTTGG ATTGGAGGTA TTGGCAACGC CTTCCATCAA GACAAGCAAA
 3401 AGGAAAATGC AGGATTCCTG TTGATTTCCTA GAGGTTATAT TGTTGGTGGC
 5 3451 AGCATGACCA CCCCTCAAGA ATATACCTTT GCTGTTGCAT TCAGCCAACT
 3501 CTTTGGCAAA TCTAAGGATT ACGTAGTCTC GGATATTAAA TCTCAAGTCT
 3551 ATGCAGGATC TCTCTGTGCT CAGAGCTCTT ATGTCATTCC CCTGCATAGC
 3601 TCATTACGTC GCCACGTCCT CTCTAAGGTC CTTCCAGAGC TCCCAGGAGA
 3651 AACTCCCTCT GTTCTCCATG GTCAAGTTTC CTATGGAAGA AACCACCATA
 10 3701 ATATGACGAC AAAGCTTGCG AACAAACAC AAGGGAATC AGACTGGGAC
 3751 AGCCATAGCT TCGCTGTTGA AGTCGGTGGT TCTCTTCCTG TAGATCTAAA
 3801 CTACAGATAC CTTACCAGCT ACTCTCCCTA TGTGAAACTC CAAGTTGTGA
 3851 GTGTAAATCA AAAAGGATTC CAAGAGGTTG CTGCTGATCC ACGTATCTTT
 3901 GACGCTAGCC ATCTGGTCAA CGTGTCTATC CCTATGGGAC TCACCTTCAA
 3951 ACACGAATCA GCAAAGCCCC CCAGTGCTTT GCTTCTTACT TTAGGTTACG
 15 4001 CTGTAGATGC TTACCGGGAT CACCCTCACT GCCTGACCTC CTTAACAAAT
 4051 GGCACCTCGT GGTCTACGTT TGCTACAAAC TTATCACGAC AAGCTTTCTT
 4101 TGCTGAGGCT TCTGGACATC TGAAGTTACT TCATGGTCTT GACTGCTTCG
 4151 CTTCTGGAAG TTGTGAAGTG CGCAGCTCCT CAAGAAGCTA TAATGCAAA
 4201 TGTGGAATC GTTATTCTTT CTAA

20 The PSORT algorithm predicts an outer membrane location (0.915).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 16A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 16B) and for FACS analysis (Figure 16C). A GST-fusion protein was also expressed.

The cp6727 protein was also identified in the 2D-PAGE experiment (Cpn0444).

25 These experiments show that cp6727 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 17

The following *C.pneumoniae* protein (PID 4376731) was expressed <SEQ ID 33; cp6731>:

1 MKSSLHWFLI SSSLALPLSL NFSAFAAVVE INLGPTNSFS GPGTYTPPAQ
 30 51 TTNADGTIYN LTGDIVSITNA GSPTALTASC FKETTGNLSF QGHGYQFLLO
 101 NIDAGANCTF TNTAANKLLS FSGPSYLSLI QTTNATTGTG AIKSTGACSI
 151 QSNYSCYFGQ NFSNDNGGAL QGSSISLSLN PNLTFANKKA TQKGGALYST
 201 GGITINNTLN SASFSENTAA NNGGAIYTEA SSFISSNKAI SFINNSVTAT
 35 251 SATGAIYCS STSAPKPVLT LSDNGELNFI GNTAITSGBA IYTDNLVLS
 301 GGPTLFPKNS AIDTAAPLGG AIAIADSGSL SLSALGGDIT FEGNTVVKGA
 351 SSSQTPTRNS INIGNTNAKI VQLRASQNT IYFYDPITTS ITAALSDALN
 401 LINGPLAGNP AYQGTIVFSG EKLSEAEAAE ADNLKSTIQQ PLTLAGGQLS
 451 LKSGVTLVAK SFSQSPGSTL LMDAGTLET ADGITINNLV LNVDLSKETK
 501 KATLKATQAS QTVTLSGSL LVDPSGNVYE DVSWNNPQVF SCLTLTADDP
 40 551 ANIHITDLAA DPLEKNPIHW GYQGNWALSW QEDTATKSKA ATLTWTKTG
 601 NPNPERRGTL VANTLWGSFV DVRSIQQLVA TKVRQSQETR GIWCEGISNF
 651 PHKDSKINK GFRHISAGYV VGATTTLASD NLITAAFCQL FGKDRDHFIN
 701 KNRASAYAAS LHLQHLATLS SPSLLRYLPG SBSEQPVLFD AQISYIYSKN
 751 TMKTYTQAP KGESSWYNDG CALELASSLP HTALSHEGLF HAYFPFIKVE
 45 801 ASYIHQDSFK ERNTTLVRSF DSGDLINVSF PIGITFERFS RNERASYEAT
 851 VIYVADVYRK NPDCTTALLI NNTSWKTGT NLSRQAGIGR AGIFYAFSPN
 901 LEVTSNLSME IRGSSRSYNA DLGGKPFQF*

A predicted signal peptide is highlighted.

The cp6731 nucleotide sequence <SEQ ID 34> is:

50 1 ATGAAATCCT CTCTTCATTG GTTTTAAATC TCGTCATCTT TAGCACTTCC
 51 CTTGTCACTA AATTTCTCTG CGTTTGCTGC TGTGTTGAA ATCAATCTAG
 101 GACCTACCAA TAGCTTCTCT GGACCAGGAA CCTACACTCC TCCAGCCCAA
 151 ACAACAAATG CAGATGGAAC TATCTATAAT CTAACAGGGG ATGCTCTCAAT
 201 CACCAATGCA GGATCTCCGA CAGCTCTAAC CGCTTCCTGC TTAAAGAAA

251 CTACTGGGAA TCTTTCTTTC CAAGGCCACG GCTACCAATT TCTCCTACAA
 301 AATATCGATG CGGGAGCGAA CTGTACCTTT ACCAATACAG CTGCAAATAA
 351 GCTTCTCTCC TTTTCAGGAT TCTCCTATTT GTCACATAA CAAACCACGA
 401 ATGCTACCAC AGGAACAGGA GCCATCAAGT CCACAGGAGC TTGTCTTATT
 5 451 CAGTCGAACT ATAGTTGCTA CTTTGGCCAA AACTTTTCTA ATGACAATGG
 501 AGGCGCCCTC CAAGGCAGCT CTATCAGTCT ATCGCTAAAC CCCAACCTAA
 551 CGTTTGCCAA AAACAAAGCA ACGCAAAAAG GGGGTGCCCT CTATTCCACG
 601 GGAGGGATTA CAATTAACAA TACGTTAAAC TCAGCATCAT TTTCTGAAAA
 10 651 TACCGCGGCG AACAAATGGCG GAGCCATTTA CACGGAAGCT AGCAGTTTTA
 701 TTAGCAGCAA CAAAGCAATT AGCTTTATAA ACAATAGTGT GACCGCAACC
 751 TCAGCTACAG GGGGAGCCAT TTACTGTAGT AGTACATCAG CCCCCAAACC
 801 AGCTTTAACT CTATCAGACA ACGGGGAAC TGAACCTTATA GGAAATACAG
 851 CAAATCTAGT TGGTGGGGCG ATTTATACTG ACAATCTAGT TCTTTCTTCT
 15 901 GGAGGACCTA CGCTTTTAA AAACAACCTCT GCTATAGATA CTGCAGCTCC
 951 CTTAGGAGGA GCAATTGCCA TTGCTGACTC TGGATCTTTG AGTCTTTCGG
 1001 CTCTTGGTGG AGACATCACT TTTGAAGGAA ACACAGTAGT CAAAGGAGCT
 1051 TCTTTCGAGTC AGACCACTAC CAGAAATTCT ATTAACATCG GAAACACCAA
 1101 TGCTAAGATT GTACAGCTGC GAGCCTCTCA AGGCAATACT ATCTACTTCT
 1151 ATGATCCTAT AACAACTAGC ATCACTGCAG CTCTCTCAGA TGCTCTAAAC
 20 1201 TTAAATGGTC CTGACCTTGC AGGGAATCCT GCATATCAAG GAACCATCGT
 1251 ATTTTCTGGA GAGAAGCTCT CGGAAGCAGA AGCTGCAGAA CTGTATAATC
 1301 TCAAATCTAC AATTTCAGCAA CCTCTAACTC TTGCGGGAGG GCAACTCTCT
 1351 CTTAAATCAG GAGTCACTCT AGTTGCTAAG TCCTTTTCGC AATCTCCGGG
 1401 CTCTACCTCT CTCATGGATG CAGGGACCAC ATTAGAAACC GCTGATGGGA
 25 1451 TCACATCAA TAATCTTGT CTCAATGTAG ATTCTTAAA AGAGACCAAG
 1501 AAGGCTACGC TAAAAGCAAC ACAAGCAAGT CAGACAGTCA CTTTATCTGG
 1551 ATCGCTCTCT CTGTAGATC CTTCTGGAAA TGTCTACGAA GATGTCTCTT
 1601 GGAATAACCC TCAAGTCTTT TCTTGTCTCA CTCCTACTGC TGACGACCCC
 1651 GCGAATATTC ACATCACAGA CTTAGCTGCT GATCCCTTAG AAAAAATCC
 30 1701 TATCCATTGG GGATACCAAG GGAATTGGGC ATTATCTTGG CAAGAGGATA
 1751 CTGCGACTAA ATCCAAAGCA GCGACTCTTA CCTGGACAAA AACAGGATAC
 1801 AATCCGAATC CTGAGCGTCG TGGAACCTTA GTTGCTAACA CGCTATGGGG
 1851 ATCCTTTGTT GATGTGCGCT CCATACAACA GCTTGTAGCC ACTAAAGTAC
 1901 GCCAATCTCA AGAACTCGC GGCACTGGT GTGAAGGGAT CTCGAACCTC
 35 1951 TTCCATAAAG ATAGCACGAA GATAAATAAA GGTTTTCGCC ACATAAGTGC
 2001 AGGTATGTT GTAGGAGCGA CTACAACATT AGCTTCTGAT AATCTTATCA
 2051 CTGCAGCCTT CTGCCAATTA TTCGGGAAAG ATAGAGATCA CTTTATAAAT
 2101 AAAAATAGAG CTTCTGCCTA TGCAGCTTCT CTCCATCTCC AGCATCTAGC
 2151 GACCTTGTCT TCTCCAAGCT TGTACGCTA CCTTCTGGA TCTGAAAGTG
 40 2201 AGCAGCCTGT CCTCTTGAT GCTCAGATCA GCTATATCTA TAGTAAAAAT
 2251 ACTATGAAAA CCTATTACAC CCAAGCACCA AAGGAGAGA GCTCGTGGTA
 2301 TAATGACGGT TGCCTCTGG AACTTGCAG CTTCCCTACCA CACACTGCTT
 2351 TAAGCCATGA GGGTCTCTTC CACGCGTATT TTCCCTTCAT CAAAGTAGAA
 45 2401 GCTTCGTACA TACACCAAGA TAGCTTCAAA GAACGTAATA CTACCTTGGT
 2451 ACGATCTTTC GATAGCGGTG ATTTAATTAA CGTCTCTGTG CCTATTGGAA
 2501 TTACCTTCGA GAGATTCTCG AGAAACGAGC GTGCGTCTTA CGAAGCTACT
 2551 GTCATCTACG TTGCCGATGT CTATCGTAAG AATCCTGACT GCACGACAGC
 2601 TCTCTAATC AACAAATACCT CGTGGAAAAC TACAGGAACG AATCTCTCAA
 2651 GACAAGCTGG TATCGGAAGA GCAGGGATCT TTTATGCCTT CTCTCCAAAT
 50 2701 CTTGAGGTCA CAAGTAACCT ATCTATGGAA ATTCTGGAT CTTACGCGAG
 2751 CTACAATGCA GATCTTGAG GTAAGTTCCA GTTCTAA

The PSORT algorithm predicts an outer membrane location (0.926).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 17A. A GST-fusion protein was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 17B; his-tag) and for FACS analysis (Figure 17C; his-tag and GST-fusion).

The GST-fusion protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis. Less cross-reactivity was seen with the his-fusion.

These experiments show that cp6731 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 18

The following *C.pneumoniae* protein (PID 4376737) was expressed <SEQ ID 35; cp6737>:

```

5      1  MPLSPKSSSF CLLACLCSAS CAPAETRLGG NRVPPITNQG EEILLTSDFV
      51  CSNFLGASFS SSFINSSSNL SLLGKGLSLT PTSCQAPTNS NYALLSAAET
     101  LTFKNFSSIN FTGNQSTGLG GLIYKDIVF QSIKDLIFTT NRVAYSPASV
     151  TTSATPAITT VTTGASALQP TDSLTVENIS QSIKFPGNLA NFGSAISSSP
     201  TAVVVFVSLT QGGSRLVIFYD PITHSLPTTS PSNKDITINA NGASGSVVFT
10      251  NSLKGVTPESS GTYALGSGGA ICIPGTPEL KNNQKCTFS YNGTPNDAGA
     301  IYAETCNIVG NQGALLLDSN TAARNNGAIC AKVLNIQGRG PIEFSRNRAE
     351  KGAIFIGPS VGDPAKQST LTIASEGDI AFQGNMLNTK PGIRNAITVE
     401  AGGEIVLSLA QGGSRLVIFYD PITHSLPTTS PSNKDITINA NGASGSVVFT
     451  SKGLSSTELL LPANTTTILL GTVKIASGEL KITDNAVNVN LGFATQSGSQ
15      501  LTLGSGGTGLG LATPTGAPAA VDFTIGKLAF DPFSFLKRFV VSASVNAGTK
     551  NVTLTGALVL DEHDVTDLYD MVSLQTFVAI PIAVFKGATV TKTGFPDGEI
     601  ATPSHYGYQG KWSYTSRPL LIPAPDGGFP GGPSPSANTL YAVWNSDTLV
     651  RSTYILDPER YGEIVSNLW ISFLGNQAFS DILQDVLLID HPGLSITAKA
     701  LGAYVEHTPR QGHEGFSGRY GGYQAALSMN YTDHTTLGLS FGQLYKGTNA
20      751  NPYDSRCSEQ MYLLSFFGQF PIVTQKSEAL ISWKAAYGYS KNHLNTTYLR
     801  PDKAPKSGQK WHNNSYYVLI SAEHPFLNWC LLTRPLAQAW DLSGFISAEP
     851  LGGWQSKFTE TGDLORSFSR GKGYNVSLPI GCSSQWFTPF KKAPSTLTIK
     901  LAYKPDIVRV NPHNIVTVVS NQESTSISGA NLRRHGLFVQ IHDVVDLTED
     951  TQAFNLNYTFD GKNGFTNHRV STGLKSTF*

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25 A predicted signal peptide is highlighted.

The cp6737 nucleotide sequence <SEQ ID 36> is:

```

      1  ATGCCTCTTT CTTTCAAATC TTCATCTTTT TGTCTACTTG CCTGTTTATG
     51  TAGTGCAAGT TGCGCGTTTG CTGAGACTAG ACTCGGAGGG AACTTTGTTC
    101  CTCCAATTAC GAATCAGGGT GAAGAGATCT TACTCACTTC AGATTTTGTT
30     151  TGTTCAAACT TCTTGGGGGC GAGTTTTCAT AGTTCTCTTA TCAATAGTTC
     201  CAGCAATCTC TCCTTATTAG GGAAGGGCCT TTCTTAAACG TTTACCTCTT
     251  GTCAAGCTCC TACAAATAGT AACTATGCGC TACTTTCTGC CGCAGAGACT
     301  CTGACCTTCA AGAATTTTTC TTCTATAAAC TTTACAGGGA ACCAATCGAC
     351  AGGACTTGGC GGCTCATCTT ACGGAAAAGA TATTGTTTTC CAATCTATCA
35     401  AAGATTGTAT CTTCACTACG AACCGTGTG CCTATCTCTC AGCATCTGTA
     451  ACTACGTCGG CAACTCCCGC AATCACTACA GTAACACAG GAGCCTCTGC
     501  TCTCCAACCT ACAGACTCAC TCACTGTCGA AAACATATCC CAATCGATCA
     551  AGTTTTTTGG GAACCTTGCC AACTTCGGCT CTGCAATTAG CAGTTCTCCC
     601  ACGGCAGTCG TTAAATTTCAT CAATAACACC GCTACCATGA GCTTCTCCCA
40     651  TAACCTTACT TCGTCAGGAG GCGGCGTGAT TTATGAGGGA AGCTCTCTCC
     701  TTTTGTAAAA CAATCTTGGA TGCATCATCT TCACCGCCAA CTCCTGTGTG
     751  AACAGCTTAA AAGGCGTCAC CCCTTCATCA GGAACCTATG CTTTAGGAAG
     801  TGGCGGAGCC ATCTGCATCC CTACGGGAAC TTTTGAATTA AAAACAATC
     851  AGGGGAAGTG CACCTTCTCT TATAATGGTA CACCAAATGA TGCGGCTGCG
45     901  ATCTACGCCG AAACCTGCAA CATCGTAGGG AACCAGGGTG CCTGTCTCCT
     951  AGATAGCAAC ACTGCAGCGA GAAATGGCGG AGCCATCTGT GCTAAAGTGC
    1001  TCAATATTCA AGGACCGGGT CCTATTGAAT TCTCTAGAAA CCGCGCGGAG
    1051  AAGGTTGGAG CTATTTTCAT AGGCCCTCTT GTTGGAGACC CTGCGAAGCA
    1101  AACATCGACA CTTACGATTT TGGCTTCCGA AGGTGATATT GCGTTCCAAG
50     1151  GAAACATGCT CAATACAAAA CCTGGAATCC GCAATGCCAT CACTGTAGAA
    1201  GCAGGGGGAG AGATTGTGTC TCTATCTGCA CAAGGAGGCT CACGCTTGT
    1251  ATTTTATGAT CCCATTACAC ATAGCCTCCC AACCACAAGT CCGTCTAATA
    1301  AAGACATTAC AATCAACGCT AATGGCGCTT CAGGATCTGT AGTCTTTACA
    1351  AGTAAGGGAC TCTCTCTAC AGAACTCTG TTGCCTGCCA ACACGACAAC
55     1401  TATACTTCTA GGAACAGTCA AGATCGCTAG TGGAGAACTG AAGATTACTG
    1451  ACAATGCGGT TGTCATGTTT CTTGGCTTCG CTACTCAGGG CTCAGTCCAG
    1501  CTTACCCCTG GCTCTGGAGG AACCTTAGGG CTGGCAACAC CCACGGGAGC
    1551  ACCTGCCGCT GTAGACTTTA CGATTGGAAA GTTAGCATTC GATCCTTTTT
    1601  CCTTCTTAAA AAGAGATTTT GTTTCAGCAT CAGTAAATGC AGGCACAAAA
60     1651  AACGTCACTT TAACAGGAGC TCTGTTCTT GATGAACATG ACGTTACAGA

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1701 TCTTTATGAT ATGGTGTGTCAT TACAAACTCC AGTAGCAATT CCTATCGCTG
 1751 TTTTCAAAGG AGCAACCGTT ACTAAGACAG GATTTCCCTGA TGGGGAGATT
 1801 GCGACTCCAA GCCACTACGG CTACCAAGGA AAGTGGTCCT ACACATGGTC
 1851 CCGTCCCCTG TTAATTCAG CTCCTGATGG AGGATTTCTT GGAGGTCCCT
 5 1901 CTCCTAGCGC AAATACTCTC TATGCTGTAT GGAATTCAGA CACTCTCGTG
 1951 CGTTCTACCT ATATCTTAGA TCCCGAGCGT TACGGAGAAA TTGTCAGCAA
 2001 CAGCTTATGG ATTTCTTCT TAGGAAATCA GGCATTTCTT GATATTTCTC
 2051 AAGATGTTCT TTTGATAGAT CATCCCGGGT TGTCCATAAC CGCGAAAGCT
 2101 TTAGGAGCCT ATGTCGAACA CACACCAAGA CAAGGACATG AGGGCTTTTC
 10 2151 AGGTGCGCTAT GGAGGCTACC AAGCTGCGCT ATCTATGAAC TACACGGACC
 2201 ACACCTACGTT AGGACTTTCT TTCGGGCAGC TTTATGGAAA AACTAACGCC
 2251 AACCCTACG ATTCACGTTG CTCAGAACAA ATGTATTTAC TCTCGTTCTT
 2301 TGGTCAATTC CCTATCGTGA CTCAAAAGAG CGAGGCCTTA ATTTCTTGGA
 2351 AAGCAGCTTA TGGTTATTCC AAAAATCACC TAAATACCAC CTACCTCAGA
 15 2401 CCTGACAAAG CTCAAAATC TCAAGGGCAA TGGCATAACA ATAGTTACTA
 2451 TGTTCTTAT TCTGCAGAAC ATCTTTCTT AAACCTGGTG CTTCCTACAA
 2501 GACCTCTGGC TCAAGCTTGG GATCTTTTCT GTTTTATTTT CGCAGAATTC
 2551 CTAGGTGCTT GGCAAAGTAA GTTCACAGAA ACTGGAGATC TGCAACGTAG
 20 2601 CTTTAGTAGA GGTAAAGGGT ACAATGTTTC CCTACCGATA GGATGTTCTT
 2651 CTCAATGGTT CACACCATT AAGAAGGCTC CTTCCTACACT GACCATCAAA
 2701 CTTGCCTACA AGCCTGATAT CTATCGTGT CACCTCACA ATATTGTGAC
 2751 TGTCTGTCTA AACCAAGAGA GCACCTCGAT CTCAGGAGCA AATCTACGCC
 2801 GCCACGGTTT GTTTGTACAA ATCCATGATG TAGTAGATCT CACCGAGGAC
 2851 ACTCAGGCCT TTCTAAACTA TACCTTTGAC GGGAAAAATG GATTTACAAA
 25 2901 CCACCGAGTG TCTACAGGAC TAAATCCAC ATTTTAA

The PSORT algorithm predicts an outer membrane location (0.940).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 18A. The recombinant protein was used to immunise mice, whose sera were used in an immunoblot analysis blot (Figure 18B) and for FACS analysis (Figure 18C). A his-tagged protein was also expressed.

The cp6737 protein was also identified in the 2D-PAGE experiment (Cpn0454) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6737 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

35 Example 19

The following *C.pneumoniae* protein (PID 4377090) was expressed <SEQ ID 37; cp7090>:

1 MNIHSLWKLC TLALLALPA CSLSPNYGWE DSCNFTCHHTR RKKPSSFGFV
 51 PLYTEEDFNP NPTFGYDSEK EEKQYKSSQV AAFRNITFAT DSYTIKGEEN
 101 LAILTNLVHY MKKNPKATLY IEGHTDERGA ASYNLALGAR RANAIKEHLR
 40 151 KQGISADRLS TISYGKEHPL NSGHNELAWQ QNRRTEFKIH AR*

A predicted signal peptide is highlighted.

The cp7090 nucleotide sequence <SEQ ID 38> is:

1 ATGAATATAC ATTCCCTATG GAAACTTTGT ACTTTATTGG CTTTACTTGC
 51 ATTGCCAGCA TGAGCCCTTT CCCCTAATTA TGGCTGGGAG GATTCTGTGA
 45 101 ATACATGCCA TCATACAAGA CGAAAAAAGC CTTCTTCTTT TGGCTTTGTT
 151 CCTCTCTATA CCGAAGAGGA CTTTAACCCT AATTTTACCT TCGGTGAGTA
 201 TGATTCCAAA GAAGAAAAAC AATACAAATC AAGCCAAGTT GCAGCATTTT
 251 GTAATATCAC CTTTGCTACA GACAGCTATA CAATTAAAGG TGAAGAGAAC
 301 CTTGCGATTG TCACGAACCT GGTTCACCTAC ATGAAGAAAA ACCCGAAAGC
 50 351 TACACTGTAC ATTGAAGGGC ATACTGACGA GCGTGGAGCT GCATCCTATA
 401 ACCTTGCTTT AGGAGCACGA CGAGCCAATG CGATTAAAGA GCATCTCCGA
 451 AAGCAGGGAA TCTCTGACGA TCGTCTATCT ACTATTTCTT ACGGAAAAAG

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501 ACATCCTTTA AATTCGGGAC ACAACGAACT AGCATGGCAA CAAAATCGCC
 551 GTACAGAGTT TAAGATTCAT GCACGCTAA

The PSORT algorithm predicts an outer membrane location (0.790).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 19A.

- 5 A his-tagged protein was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 19B) and for FACS analysis.

These experiments show that cp7090 is useful immunogen. These properties are not evident from the sequence alone.

Example 20

- 10 The following *C.pneumoniae* protein (PID 4377091) was expressed <SEQ ID 39; cp7091>:

1 MLROLCFQVF FFCFASLVYA EELEVVVRSE HITLPIEVSC QTDTKDPKIQ
 51 KYLSSLTEIF CKDIALGDCL QPTAASKESS SPLAISLRH VPQLSVVLLQ
 101 SSKTPQTLCS FTISQNLSD RQKIHAADT VHVALTGIPG ISAGKIVPAL
 151 SSLGKDQKLE QGELWTFDYD GKNLAPLTFE CSLSITPKWV GVGSNFPYLY
 15 VSYKYGVPKI FLGSLENTG KKVLPKGNQ LMPTFSRKK LLAFVADTYG
 201 NPDLFIQFFS LTSGPMGRPR RLLNENFGTQ GNPSFNPEGS QLVFISNKDG
 251 RPRLYIMSLD PEPQAPRLLT KKYRNSSCPA WSPDGKKIAF CSVIKGVRQI
 301 CIYDLSSGED YQLTTSPTNK ESPSWAIDSR HLVFSAGNAE ESELYLISLV
 351 TKKTNKIAIG VGEKRFPSWG AFPQQPIKRT L*

- 20 A predicted signal peptide is highlighted.

The cp7091 nucleotide sequence <SEQ ID 40> is:

1 ATGTTACGGC AACTATGCTT CCAAGTFTTT TTCTTTTGCT TCGCATCGCT
 51 AGTCTATGCT GAAGAATTAG AAGTTGTGT CCGTTCCGAA CATATCACGC
 101 TCCCTATTGA GGTCTCTTGC CAGACCGATA CGAAAGATCC AAAAATACAG
 25 AAATACCTCA GCTCGCTAAC GGAGATATTT TGCAAGGACA TTGCCCTAGG
 151 AGATTGTCTA CAACCCACAG CGGCTTCTAA AGAATCGTCA TCTCCTTAG
 201 CAATATCTTT ACGGTTCAT GTACCTCAGC TATCTGTAGT GCTTTTACAG
 251 TCTTCAAAAA CTCCTCAAAC CTTATGTTCT TTTACTATTT CTCAAAATCT
 301 TTCTGTAGAT CGTCAAAAAA TCCATCACGC TGCTGTATACA GTTCATTACG
 351 CCCTCACAGG GATTCTTGGA ATCAGTGCTG GGAAAATTGT TTTTGCTCTA
 401 AGTTCTTTAG GAAAAGATCA AAAGCTCAAG CAAGGAGAAT TATGGACTAC
 451 AGATTACGAT GGGAAAAACC TCGCCCTTTT AACCACAGAA TGTTCGCTCT
 501 CTATAACTCC AAAATGGGTG GGTGTGGGAT CAAATTTTCC CTATCTCTAT
 551 GTTTCGTATA AGTATGGTGT GCCTAAATTT TTTCTTGGTT CCCTAGAGAA
 601 CACTGAAGGT AAAAAAGTCC TTCCGTTAAA AGGCAACCAA CTCATGCCTA
 35 651 CACTGAAGGT AAAAAAGTCC TTCCGTTAAA AGGCAACCAA CTCATGCCTA
 701 CGTTTTCTCC AAGAAAAAAG CTTTGTAGCT TCGTTGCTGA TACGTATGGA
 751 AATCCTGATT TATTTATTCA ACCGTTCTCA CTAACCTCAG GACCTATGGG
 801 TCGCCACGCT CGCCTCCTTA ATGAGAATTT CGGGACTCAA GGGAAATCCCT
 851 CCTTCAACCC TGAAGGATCC CAGCTTGTCT TTATATCGAA CAAAGACGGC
 40 901 CGTCCGCGTC TTTATATTAT GTCCCTCGAT CCTGAACCCC AAGCACCTCG
 951 CTTGCTGACA AAAAAATACA GAAATAGCAG TTGCCCTGCA TGGTCTCCAG
 1001 ATGGTAAAAA AATAGCCTTC TGCTCTGTAA TTAAAGGGGT GCGACAAATT
 1051 TGTATTTCAG ATCTCTCCTC TGGAGAGGAT TACCAACTCA CTACGTCTCC
 1101 CACAAATAAA GAGAGTCCTT CTTGGGCTAT AGACAGCCGT CATCTTGTCT
 45 1151 TTAGTGCGGG GAATGCTGAA GAATCAGAGT TATATTTAAT CAGTCTAGTC
 1201 ACCAAAAAAA CTAACAAAT TGCTATAGGA GTAGAGAGAA AACGGTTCCC
 1251 CTCTTGGGGT GCTTTCCTC AGCAACCGAT AAAGAGAACA CTATGA

The PSORT algorithm predicts an inner membrane location (0.109).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 20A.

- 50 A his-tagged protein was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 20B) and for FACS analysis.

These experiments show that cp7091 is a useful immunogen. These properties are not evident from the sequence alone.

Example 21

The following *C. pneumoniae* protein (PID 4376260) was expressed <SEQ ID 41; cp6260>:

```

5      1  MRFSLCGFPL VFSFTLLSVF DTSLSATTTIS LTPEDSFHGD SQNAERSYNV
      51  QAGDVYSLTG DVSISNVDNS ALNKACFNVT SGSVTFAGNH HGLYFNMISS
     101  GTTKEGAVLC CQDPQATARF SGFSTLSFIQ SPGDIKEQGC LYSKNALMLL
     151  NNYVVRFEQN QSKTKGGAIS GANVTIVGNY DSVSFYQNA TFGGAIHSSG
     201  PLQIAVNQAE IRFAQNTAKN GSGGALYS DG DIDIDQNAVY LFRNEALTT
    10  251  AIGKGGAVCC LPTSGSSTPV PIVTFSDNQ LVFERNHSIM GGGATYARKL
      301  SISSGGPTLF INNISYANSQ NLGGAIAIDT GGEISLSAEK GTITFQGNRT
      351  SLPFLNGIHL LQNAKFLKLQ ARNGYSIEFY DPITSEADGS TQLNINGDPK
      401  NKBYTGTLIF SGEKSLANDP RDFKSTIPQN VNLSAGYLVI KEGAEVTVSK
      451  FTQSPGSHLV LDLGTKLIAS KEDIAITGLA IDIDLSSSSS TAAVIKANTA
    15  501  NKQISVTD SI ELISPTGNAY EDLRMRNSQT FPLLSLEPGA GGSVTVTAGD
      551  FLFPVSPHYGF QGNWKLAWTG TGNKVGEFFW DKINYKPRPE KEGNLVPNIL
      601  WGNAVDVRS L MQVQETHASS LQTDRLWID GIGNFFHVSA SEDNIRYRHN
      651  SGGYVLSVNN EITPKHYTSM AFSQLFSRDK DYAVSNNEYR MYLGSYLYQY
      701  TTS LGNIFRY ASRNPVN NVG ILSRRFLQNP LMIFHFLCAY GHATNDMKT D
    20  751  YANFPMVKNS WRNNCWAIEC GGSMPLLVFE NGRLFQGAIP FMKLQLVYAY
      801  QGDFKETAD GRRFSNGSLT SISVPLGIRF EKLALSQDVL YDFSFSYIPD
      851  IFRKDPSC EA ALVISGDSWL VPAAHVSRHA FVSGGTGRYH FNDYTELLCR
      901  GSIECRPHAR NYNINCSKF RF*

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A predicted signal peptide is highlighted.

25 The cp6260 nucleotide sequence <SEQ ID 42> is:

```

      1  ATGCGATTMT CGCTCTGCGG ATTTCTCTCTA GTTTTCTCTT TTACATTGCT
      51  CTCAGTCTTC GACACTTCTT TGAGTGCTAC TACGATTTCT TTAACCCCAG
     101  AAGATAGTTT TCATGGAGAT AGTCAGAATG CAGAACGTTC TTATAATGTT
     151  CAAGCTGGGG ATGCTCTATAG CCTTACTGGT GATGCTCTCA TATCTAACGT
     201  CGATAACTCT GCATTAAATA AAGCCTGCTT CAATGTGACC TCAGGAAGTG
     251  TGACGTTTCG AGGAAATCAT CATGGGTAT ATTTTAATAA TATTTCTCTCA
     301  GGAACCTACAA AGGAAGGGGC TGTACTTTGT TGCCAAGATC CTCAGCAAC
     351  GGCACGTTTT TCTGGGTTCT CCACGCTCTC TTTTATTTCAG AGCCCCGGAG
     401  ATATTAAAGA ACAGGGATGT CTCTATTCAA AAAATGCACT TATGCTCTTA
    35  451  AACAATTATG TAGTGCGTTT TGAACAAAAC CAAAGTAAGA CTAAAGCGCG
      501  AGCTATTAGT GGGCGGAATG TTACTATAGT AGGCAACTAC GATTCCGTCT
      551  CTTTCTATCA GAATGCAGCC ACTTTTGAG GTGCTATCCA TTCTTCAGGT
      601  CCCCTACAGA TTGCAGTAAA TCAGGCAGAG ATAAGATTGT CACAAAATAC
      651  TGCCAAGAAT GGTCTCGGAG GGGCTTTGTA CTCCGATGGT GATATTGATA
    40  701  TTCTTCAGAA TGCTTATGTT CTATTTCGAG AAAATGAGGC ATTGACTACT
      751  GCTATAGGTA AGGGAGGGGC TGTCTGTTGT CTTCCCACTT CAGGAAGTAG
      801  TACTCCAGTT CCTATTGTGA CTTTCTCTGA CAATAACAG TTAGTCTTTG
      851  AAAGAAACCA TTCCATAATG GGTGGCGGAG CCATTTATGC TAGGAAACTT
      901  AGCATCTCTT CAGGAGGTCC TACTCTATTT ATCAATAATA TATCATATGC
    45  951  AAAITCGCAA AATTAGGTG GAGCTATTGC CATTGATACT GGAGGGGAGA
     1001  TCAGTTTATC AGCAGAGAAA GGAACAATTA CATTTCAAGG AAACCGGACG
     1051  AGCTTACCGT TTTTGAATGG CATCCATCTT TTACAAAATG CTAAATTCCT
     1101  GAAATTACAG GCGAGAAATG GATACTCTAT AGAATTTTAT GATCCTATTA
     1151  CTTCTGAAGC AGATGGGTCT ACCCAATTGA ATATCAACGG AGATCCTAAA
    50  1201  AATAAAGAGT ACACAGGGAC CATACTCTTT TCTGGAGAAA AGAGTCTAGC
      1251  AAACGATCCT AGGGATTTTA AATCTACAAT CCCTCAGAAC GTCAACCTGT
      1301  CTGCAGGATA CTTAGTTATT AAAGAGGGGG CCGAAGTCAC AGTTTCAAAA
      1351  TTCACGCAGT CTCCAGGATC GCATTAGTTT TTAGATTTAG GAACCAAACT
     1401  GATAGCCTCT AAGGAAGACA TTGCCATCAC AGGCCTCGCG ATAGATATAG
    55  1451  ATAGCTTAAG CTCATCTCTA ACAGCAGCTG TTATTAAAGC AAACACCGCA
      1501  AATAAACAGA TATCCGTGAC GGACTCTATA GAACCTATCT CGCCTACTGG
      1551  CAATGCCTAT GAAGATCTCA GAATGAGAAA TTCACAGACG TTCCCTCTGC
      1601  TCTCTTAGA GCCTGGAGCC GGGGGTAGTG TGAAGTAAAC TGCTGGAGAT
      1651  TTCTTACCGG TAAGTCCCCA TTATGGTTTT CAAGGCAATT GGAAATTAGC
    60  1701  TTGGACAGGA ACTGGAAACA AAGTTGGAGA ATTCTTCTGG GATAAAATAA

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1751 ATTATAAGCC TAGACCTGAA AAAGAAGGAA ATTTAGTTCC TAATATCTTG
 1801 TGGGGGAATG CTGTAGATGT CAGATCCTTA ATGCAGGTTT AAGAGACCCA
 1851 TGCATCGAGC TTACAGACAG ATCGAGGGCT GTGGATCGAT GGAATTGGGA
 5 1901 ATTTCTFCCA TGTATCTGCC TCCGAAGACA ATATAAGGTA CCGTCATAAC
 1951 AGCGGTGGAT ATGTTCTATC TGTAAATAAT GAGATCACAC CTAAGCACTA
 2001 TACTTCGATG GCATTTTCCC AACTCTTTAG TAGAGACAAG GACTATGCGG
 2051 TTTCCAACAA CGAATACAGA ATGTATTTAG GATCGTATCT CTATCAATAT
 2101 ACAACCTCCC TAGGGAAATAT TTTCGGTTAT GCTTCGCGTA ACCCTAATGT
 10 2151 AAACGTCGGG ATTCTCTCAA GAAGGTTTCT TCAAAATCCT CTTATGATTT
 2201 TTCATTTTTT GTGTGCTTAT GGTCA TGCCA CCAATGATAT GAAAACAGAC
 2251 TACGCAAAAT TCCCTATGGT GAAAACAGC TGGAGAAACA ATTGTTGGGC
 2301 TATAGAGTGC GGAGGGAGCA TGCCTCTATT GGTATTTGAG AACGGAAGAC
 2351 TTTTCCAAGG TGCCATCCCA TTTATGAAAC TACAATTAGT TTATGCTTAT
 15 2401 CAGGGAGATT TCAAAGAGAC GACTGCAGAT GGCCGTAGAT TTAGTAATGG
 2451 GAGTTTAAACA TCGATTTCTG TACCTCTAGG CATACGCTTT GAGAAGCTGG
 2501 CACTTTCTCA GGATGTACTC TATGACTTTA GTTTCCTCTA TATTCCTGAT
 2551 ATTTTCCGTA AGGATCCCTC ATGTGAAGCT GCTCTGGTGA TTAGCGGAGA
 2601 CTCCTGGCTT GTTCCGGCAG CACACGTATC AAGACATGCT TTTGTAGGGA
 2651 GTGGAACGGG TCGGTATCAC TTTAACGACT ATACTGAGCT CTTATGTCGA
 20 2701 GGAAGTATAG AATGCCGCCC CCATGCTAGG AATTATAATA TAAACTGTGG
 2751 AAGCAAATTT CGTTTTTAG

The PSORT algorithm predicts an outer membrane location (0.921).

The protein was expressed in *E.coli* and purified both as a his-tag and GST-fusion product. The GST-
 fusion is shown in Figure 21A. This recombinant protein was used to immunise mice, whose sera
 25 were used in a Western blot (Figure 21B) and for FACS analysis (Figure 21C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6260 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

30 Example 22

The following *C.pneumoniae* protein (PID 4376456) was expressed <SEQ ID 43; cp6456>:

1 MSSPVNNTPS APNIPAPPT TPGIPTTKPR SSFIEKVIIV AKYILFAIAA
 51 TSGALGTLIG LSGALTPGIG IALLVIFVSV MVLGLILKLD SISGGEERRRL
 101 REEVSRFTSE NQRLTVITTT LETEVKDLKA AKDQLTLEIE AFRNENGNLK
 35 151 TTAEDLEEQV SKLSEQLLEAL ERINQLIQAN AGDAQEISSE LKKLISGWDS
 201 KVVEQINTSI QALKVLLQGE WVQEAQTHVK AMQEIQIALQ AEILGMHNQS
 251 TALQKSVENL LVQDQALTRV VGELLESENK LSQACSALRQ EIEKLAQHET
 301 SLQQRIDAML AQEQNLAEQV TALEKMKQEA QKAESEFIAC VRDRTFGRRE
 351 TPPPTFPVVE GDESQBEDEG GTPPVSQPS S FVD RATGDGQ *

40 The cp6456 nucleotide sequence <SEQ ID 44> is:

1 ATGTCATCTC CTGTAAATAA CACACCCTCA GCACCAAACA TTCCAATACC
 51 AGCGCCACAG ACTCCAGGTA TTCCTACAAC AAAACCTCGT TCTAGTTTCA
 101 TTGAAAAGGT TATCATTGTA GCTAAGTACA TACTATTTGC AATTGCAGCC
 45 151 ACATCAGGAG CACTCGGAAC AATTCTAGGT CTATCTGGAG CGCTAACCCC
 201 AGGAATAGGT ATTGCCCTTC TTGTTATCTT CTTTGTCTCT ATGGTGCTTT
 251 TAGGTTTAAT CCTTAAAGAT TCTATAAGTG GAGGAGAAGA ACGCAGGCTC
 301 AGAGAAGAGG TCTCTCGATT TACAAGTGAG AATCAACGGT TGACAGTCAT
 351 AACCACAACA CTTGAGACTG AAGTAAAGGA TTTAAAAGCA GCTAAAGATC
 401 AACTTACACT TGAATTCGAA GCATTTAGAA ATGAAAACGG TAATTTAAAA
 50 451 ACAACTGCTG AGGACTTAGA AGAGCAGGTT TCTAAACTTA GCGAACAAAT
 501 AGAAGCACTA GAGCGAATTA ATCAACTTAT CCAAGCAAAC GCTGGAGATG
 551 CTCAAGAAAT TTCGTCTGAA CTAAAGAAAT TAATAAGCGG TTGGGATTCC
 601 AAAGTTGTTG AACAGATAAA TACTTCTATT CAAGCATTGA AAGTGTATT
 651 GGGTCAAGAG TGGGTCAAG AGGCTCAAAC ACACGTTAAA GCAATGCAAG
 55 701 AGCAAATTC A GCATTGCAA GCTGAAATTC TAGGAATGCA CAATCAATCT

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751 ACAGCATTGC AAAAGTCAGT TGAGAATCTA TTAGTACAAG ATCAAGCTCT
801 AACAAGAGTA GTAGGTGAGT TGTTAGAGTC TGAGAACAAAG CTAAGCCAAG
851 CTTGTTCTGC GCTACGTCAA GAAATAGAAA AGTTGGCCCA ACATGAAACA
901 TCTTTGCAAC AACGTATTGA TCGCATGCTA GCCCAAGAGC AAAATTGCGC
951 AGAGCAGGTC ACAGCCCTTG AAAAAATGAA ACAAGAAGCT CAGAAGGCTG
1001 AGTCCGAGTT CATTGCTTGT GTACGTGATC GAACTTTCGG ACGTCGTGAA
1051 ACACCTCCAC CAACAACACC TGTAGTTGAA GGTGATGAAA GTCAAGAAGA
1101 AGACGAAGGA GGTACTCCCC CAGTATCACA ACCATCTTCA CCCGTAGATA
1151 GAGCAACAGG AGATGGTCAG TAA

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10 The PSORT algorithm predicts inner membrane (0.127).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 22A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 22B) and for FACS analysis (Figure 22C). A his-tag protein was also expressed.

15 These experiments show that cp6456 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 23

The following *C.pneumoniae* protein (PID 4376729) was expressed <SEQ ID 45; cp6729>:

```

1 MKIPLHKLLI SSTLVTPILL SIATYGADAS LSPTDSFDGA GGSFTTPKST
51 ADANGTNYVL SGNVYINDAG KGTALTGCCF TETTGDLTFT GKGYSFSFNT
20 101 VDAGSNAGAA ASTTADKALT FTGFNSLSFI AAPGTTVASG KSTLSSAGAL
151 NLTDNGTILF SQNVSNNEANN NGGATITKTL SISGNTSSIT FTSNSAKKLG
201 GAIYSSAAAS ISGNTGQLVF MNNKGETGGG ALGFEASSSI TQNSSLFFSG
251 NTATDAAGKG GAIYCEKTGE TPTLTISGNK SLTFAENSSV TQGGATCAHG
301 LDLSAAGPTL FSNNRGNTA AGKGGALALA DSGSLSLSAN QGDIITFLGNT
25 351 LTSTSAPTST RNAIYLGSSA KITNLRAAQG QSIYFYDPIA SNTTGASDVL
401 TINQPSNSP LDYSGTIVFS GEKLSADEAK AADNPTSILK QPLALASGTL
451 ALKGNVELDV NGFTQTEGST LLMQPGTKLK ADTEAISLTR LVVDLSALEG
501 NKSVSJETAG ANKTITLTSP LVFQDSSGNF YESHTINQAF TQPLVVFTAA
551 TAASDIYIDA LLTSPVQTPE PHYGYQGHWE ATWADTSTAK SGTMTWVVTG
30 601 YNPNPERRAS VVPDSLWASF TDIRTLQQIM TSQANSIYQQ RGLWASGTAN
651 FFHKDKSGTN QAFRHKSQYGY IVGGSIEDFS ENIFSVAPCQ LFGKDKDLFI
701 VENTSHNYLA SLYLQHRAFL GGLPMPSPFGS ITDMLKDIPL ILNAQLSYSY
751 TKNDMDTRYT SYPEAQGSWT NNSGALELGG SLALYLPKRA PFFQGYFPFL
801 KFAQVYSRQQ NFKESGAEAR AFDDGDLVNC SIPVGIRLEK ISEDEKNNFE
35 851 ISLAYIGDVY RKNPRSRTSL MVSGASWTSI CKNLARQAPL ASAGSHLTL
901 PHVELSGEAA YELRGSATY NVDCGLRYSF *

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A predicted signal peptide is highlighted.

The cp6729 nucleotide sequence <SEQ ID 46> is:

```

1 ATGAAAATAC CCTTGCACAA ACTCCTGATC TCTTCGACTC TTGTCACTCC
40 51 CATTCTATTG AGCATTGCAA CTTACGGAGC AGATGCTTCT TTATCCCTTA
101 CAGATAGCTT TGATGGAGCG GCGGCTCTTA CATTTACTCC AAAATCTACA
151 GCAGATGCCA ATGGAACGAA CTATGTCTTA TCAGGAAATG TCTATATAAA
201 CGATGCTGGG AAAGGCACAG CATTAACAGG CTGCTGCTTT ACAGAACTA
251 CGGGTGATCT GACATTTACT GGAAAGGGAT ACTCATTTTC ATTCAACACG
45 301 GTAGATGCGG GTTCGAATGC AGGAGCTGCG GCAAGCACAA CTGCTGATAA
351 AGCCCTAACA TTCACAGGAT TTTCTAACCT TTCTTCAATT GCAGCTCCTG
401 GAACTACAGT TGCTTCAGGA AAAAGTACTT TAAGTTCTGC AGGAGCCTTA
451 AATCTTACCG ATAATGGAAC GATTCTCTTT AGCCAAAACG TCTCCAATGA
501 AGCTAATAAC AATGGCGGAG CGATCACCAC AAAAATCTTT TCTATTCTTG
50 551 GGAATACCTC TTCTATAACC TTCCTAGTA ATAGCGCAAA AAAATTAGGT
601 GGAGCGATCT ATAGCTCTGC GGCTGCAAGT ATTTCAAGAA ACACCGGCCA
651 GTTAGTCTTT ATGAATAATA AAGGAGAAAC TGGGGGTGGG GCTCTGGGCT
701 TTGAAGCCAG CTCCTCGATT ACTCAAAATA GCTCCCTTTT CTTCTCTGGA
751 AACACTGCAA CAGATGCTGC AGGCAAGGGC GGGGCCATTT ATTGTGAAAA
55 801 AACAGGAGAG ACTCTACTC TTAATATCTC TGGAAATAAA AGTCTGACCT
851 TCGCCGAGAA CTCCTCAGTA ACTCAAGGCG GAGCAATCTG TGCCCATGGT

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901 CTAGATCTTT CCGCTGCTGG CCCTACCCCTA TTTTCAAATA ATAGATGCGG
951 GAACACAGCT GCAGGCAAGG GCGGCGCTAT TGCAATTGCC GACTCTGGAT
1001 CTTTAAAGTCT CTCTGCAAAT CAAGGAGACA TCACGTTCCCT TGGCAACACT
1051 CTAACCTCAA CCTCCGCGCC AACATCGACA CGGAATGCTA TCTACCTGGG
1101 ATCGTCAGCA AAAATTACGA ACTTAAGGGC AGCCCAAGGC CAATCTATCT
1151 ATTTCTATGA TCCGATTGCA TCTAACACCA CAGGAGCTTC AGACGTTCTG
1201 ACCATCAACC AACCGGATAG CAACTCGCCT TTAGATTATT CAGGAACGAT
1251 TGTATTTTCT GGGGAAAAGC TCTCTGCAGA TGAAGCGAAA GCTGCTGATA
1301 ACTTCACATC TATATTAAAG CAACCATTGG CTCTAGCCTC TGGAACCTTA
1351 GCACTCAAAG GAAATGTCGA GTTAGATGTC AATGGTTTCA CACAGACTGA
1401 AGGCTCTACA CTCCTCATGC AACCAGGAAC AAAGTCAAA GCAGATACTG
1451 AAGCTATCAG TCTTACCAAA CTTGTCTGTG ATCTTTCTGC CTTAGAGGGA
1501 AATAAGAGTG TGTCCATTGA AACAGCAGGA GCCAACAAAA CTATAACTCT
1551 AACCTCTCCT CTTGTTTTCC AAGATAGTAG CGGCAATTTT TATGAAAGCC
1601 ATACGATAAA CCAAGCCTTC ACGCAGCCTT TGGTGGTATT CACTGCTGCT
1651 ACTGCTGCTA GCGATATTTA TATCGATGCG CTTCTCATT CTCCAGTACA
1701 AACTCCAGAA CCTCATTACG GGTATCAGGG ACATTGGGAA GCCACTTGGG
1751 CAGACACATC AACTGCAAAA TCAGGAACCTA TGACTTGGGT AACTACGGGC
1801 TACAACCCCTA ATCTTGAGCG TAGAGCTTCC GTAGTTCCCG ATTCAATTATG
1851 GGCATCCTTT ACTGACATTC GCACTCTACA GCAGATCATG ACATCTCAAG
1901 CGAATAGTAT CTATCAGCAA CGAGGACTCT GGGCATCAGG AACTGCGAAT
1951 TTCTTCCATA AGGATAAATC AGGAACTAAC CAAGCATTCG GACATAAAAG
2001 CTACGGCTAT ATTGTGGAG GAAGTGCTGA AGATTTTCTT GAAAATATCT
2051 TCAGTGTAGC TTTCTGCCAG CTCCTCGGTA AAGATAAAGA CCTGTTTATA
2101 GTTGAAAATA CCTCTCATAA CTATTTAGCG TCGCTATACC TGCAACATCG
2151 AGCATTCCTA GGAGGACTTC CCATGCCCTC ATTTGGAAGT ATCACCAGCA
2201 TGCTGAAAGA TATTCCTCTC ATTTTGAATG CCCAGCTAAG CTACAGCTAC
2251 ACTAAAAATG ATATGGATAC TCGCTATACT TCCTATCCTG AAGCTCAAGG
2301 CTCTTGGACC AATAACTCTG GGGCTCTAGA GCTCGGAGGA TCTCTGGCTC
2351 TATATCTCCC TAAAGAAGCA CCGTCTCTCC AGGGATATTT CCCCTTCTTA
2401 AAGTTCAGG CAGTCTACAG CCGCCAACAA AACTTTAAAG AGAGTGGCGC
2451 TGAAGCCCGT GCTTTTGATG ATGGAGACCT AGTGAAGTGC TCTATCCCTG
2501 TCGGCATTCT GTTAGAAAAA ATCTCCGAAG ATGAAAAAAA TAATTTGAGG
2551 ATTTCTCTAG CCTACATTGG TGATGTGTAT CGTAAAAATC CCCGTTGCGG
2601 TACTTCTCTA ATGGTCAATG GAGCCTCTTG GACTTCGCTA TGTAAAAACC
2651 TCGCACGACA AGCCTTCTTA GCAAGTGCTG GAAGCCATCT GACTCTCTCC
2701 CCTCATGTAG AACTCTCTGG GGAAGCTGCT TATGAGCTTC GTGGCTCAGC
2751 ACACATCTAC AATGTAGATT GTGGGCTAAG ATACTCATTC TAG
  
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The PSORT algorithm predicts outer membrane (0.927).

40 The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 23A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 23B) and for FACS analysis (Figure 23C). A his-tag protein was also expressed.

The cp6729 protein was also identified in the 2D-PAGE experiment (Cpn0446) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

45 These experiments show that cp6729 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 24

The following *C. pneumoniae* protein (PID 4376849) was expressed <SEQ ID 47; cp6849>:

50
 55

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1  MSKLIRRVVT VLALTSMA SC FASGGIEAAV AESLITKIVA SAETKPAPVP
51  MTAKKVRLLVR RNKQPVBEQKS RGAFCDKEFY PCEEGRCQPV EAQQESCYGR
101 LYSVKVNDDC NVEICQSVPE YATVGSYPYPI EILAIGKKDC VDVVITQQLP
151 CEAEFVSSDP ETTPTSDGKL VWKIDRLGAG DKCKITVWVK PLKEGCCFTA
201 ATVCACPELR SYTKCQPAI CIKQEGPDCA CLRCPVCYKI EVVNTGSAIA
251 RNVTVDNFVP DGYSHASGQR VLSFNLGDMR PGDKKVFTVE FCPQRRGQIT
301 NVATVTVCGG HKCSANVTTV VNEPCVQVNI SGADWSYVCK PVEYSISVSN
351 PGDLVLHDVV IQDTLPSGVT VLEAPGGEIC CNKVVWRIKE MCPGETLQFK
  
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401 LVVKAQVPGR FTNQVAVTSE SNCGTCTSCA ETTTHWKGLA ATHMCVLDTN
 451 DPICVGENTV YRICVTNRGS AEDTNVSLIL KFSKELQPIA SSGPTKGTIS
 501 GNTVVFDAIP KLGSKESVEF SVTLKGIAPG DARGEAILSS DTLTSPVSDT
 551 ENTHVY*

5 A predicted signal peptide is highlighted.

The cp6849 nucleotide sequence <SEQ ID 48> is:

1 ATGTCCAAAC TCATCAGACG AGTAGTTACG GTCCTTGCGC TAACGAGTAT
 51 GGCGAGTTGC TTTGCCAGCG GGGGTATAGA GGCCGCTGTA GCAGAGTCTC
 101 TGATTACTAA GATCGTCGCT AGTGCGGAAA CAAAGCCAGC ACCTGTTCCCT
 151 ATGACAGCGA AGAAGGTTAG ACTTGTCCGT AGAAATAAAC AACCAGTTGA
 201 ACAAATAAAGC CGTGGTGCCT TTTGTGATAA AGAATTTTAT CCCTGTGAAG
 251 AGGGACGATG TCAACCTGTA GAGGCTCAGC AAGAGTCTTG CTACGGAAGA
 301 TGTATTTCTG TAAAAGTAAA CGATGATGTC AACGTAGAAA TTTGCCAGTC
 351 CGTTCCAGAA TACGCTACTG TAGGATCTCC TTACCCCTATT GAAATCCTTG
 15 401 CTATAGGCAA AAAAGATTGT GTTGATGTTG TGATTACACA ACAGCTACCT
 451 TGCGAAGCTG AATTCGTAAG CAGTGATCCA GAAACAACCTC CTACAAGTGA
 501 TGGGAAATTA GTCTGAAAAA TCGATCGCCT GGGTGCAGGA GATAAATGCA
 551 AAATTACTGT ATGGGTAAAA CCTCTTAAAG AAGGTTGCTG CTTACAGACT
 601 GCTACTGTAT GTGCTTGCCC AGAGCTCCGT TCTTATACTA AATGCGGTCA
 20 651 ACCAGCCATT TGTAATTAAAG AAGAAGGACC TGACTGTGCT TGCCTAAGAT
 701 GCCCTGTATG CTACAAAATC GAAGTAGTGA ACACAGGATC TGCTATTGCC
 751 CGTAACGTAA CTGTAGATAA TCCTGTTCCC GATGGCTATT CTCATGCATC
 801 TGGTCAAAGA GTTCTCTCTT TTAACCTTAGG AGACATGAGA CCTGGCGATA
 851 AAAAGGTATT TACAGTTGAG TTCTGCCCTC AAAGAAGAGG TCAAATCACT
 25 901 AACGTTGCTA CTGTAACCTT CTGCGGTGGA CACAAATGTT CTGCAAATGT
 951 AACTACAGTT GTTAATGAGC CTTGTGTACA AGTAAATATC TCTGGTGCTG
 1001 ATTTGGTCTTA CGTATGTAAA CCTGTGGAGT ACTCTATCTC AGTATCGAAT
 1051 CCTGGAGACT TGGTTCTTCA TGATGTCGTG ATCCAAGATA CACTCCCTTC
 1101 TGGTGTTACA GTACTCGAAG CTCCTGGTGG AGAGATCTGC TGTAAATAAG
 30 1151 TTGTTTGGCG TATTAAAGAA ATGTGCCCAG GAGAAACCTC CCAGTTTAAA
 1201 CTTGTAGTGA AAGCTCAAGT TCCTGGAAGA TTCACAAATC AAGTTGCAGT
 1251 AACTAGTGAG TCTAACTGCG GAACATGTAC ATCTTGCCGA GAAACAACAA
 1301 CACATTGGAA AGGCTCTGCA GCTACCCATA TGTGCGTATT AGACACAAAT
 1351 GATCCTATCT GTGTAGGAGA AAATACTGTC TATCGTATCT GTGTAATAA
 35 1401 CCGTGGTTCT GCTGAAGATA CTAACGTATC TTTAATCTTG AAGTTCTCAA
 1451 AAGAACTTCA GCCAATAGCT TCTTCAGGTC CAACTAAAGG AACGATTTCA
 1501 GGTAAATACG TTGTTTTCGA CGCTTTACCT AAACTCGGTT CTAAGGAATC
 1551 TGTAAGATTT TCTGTTACCT TGAAAGGTAT TGCTCCCGGA GATGCTCGCG
 1601 GCGAAGCTAT TCTTTCTTCT GATACACTGA CTTACCAGT ATCAGACACA
 40 1651 GAAATATACC ACGTGTATTA A

The PSORT algorithm predicts periplasmic space (0.93).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 24A, and also as a his-tag protein. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 24B) and for FACS analysis (Figure 24C).

45 The cp6849 protein was also identified in the 2D-PAGE experiment (Cpn0557).

These experiments show that cp6849 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 25

The following *C.pneumoniae* protein (PID 4376273) was expressed <SEQ ID 49; cp6273>:

50 1 MGLFHLTLFG LLLCSLPISL VAKFPESVGH KILYISTQST QQALATYLEA
 51 LDAYGDHDFV VLRRIGEDYL KQSIHSSDPQ TRKSTIIGAG LAGSSEALDV
 101 LSQAMETADP LQQLVL SAV SGHLGKTSDD LLFKALASPY PVIRLEAAYR
 151 LANLKNTKVI DHLHSFIHKL PEEIQCLSAA IFLRLETEES DAYIRDLLAA
 201 KKSARSATA LQIGBYQQR FLPTLRNLLT SASPDQAEI LYALGKLKDG

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251 QSYYNIKKQL QKPDVDVTLA AAQALIALGK EEDALPVIKK QALEERPRAL
 301 YALRHLPSSEI GIPIALPIFL KTKNSEAKLN VALALLEIGC DTPKLLLEYIT
 351 ERLVQPHYNE TLALSFSGKR TLQNWKRNI IVPQDPQERE RLLSTTRGLE
 401 BQILTFLFRL PKEAYLPCIY KLLASQKTQL ATTAISFLSH TSHQEALDLL
 451 FQAAKLPGEP IIRAYADLAI YNLTKDPEKK RSLHDYAKKL IQETLLFVDT
 501 ENQRPHPSMP YLRYQVTPES RTKLMLDILE TLATSKSSED IRLLIQLMTE
 551 GDAKNFPVLA GLLIKIVE*

A predicted signal peptide is highlighted.

The cp6273 nucleotide sequence <SEQ ID 50> is:

10 1 ATGGGACTAT TCCATCTAAC TCTCTTTGGA CTTTTATGT GTAGTCTTCC
 51 CATTCTCTTT GTTGCTAAAT TCCCTGAGTC TGTAGGTCAT AAGATCCTTT
 101 ATATAAGTAC GCAATCTACA CAGCAGGCTT TAGCAACATA TCTGGAAGCT
 151 CTAGATGCCT ACGGTGATCA TGACTTCTTC GTTTTAAAGAA AAATCGGAGA
 201 AGACTATCTC AAGCAAAGCA TCCACTCCTC AGATCCGCAA ACTAGAAAAA
 15 251 GCACCATCAT TGGAGCAGGC CTGGCGGGAT CTTCAGAAGC CTTGGACGTG
 301 CTCCTCCCAAG CTATGGAAAC TGCAGACCCC CTGCAGCAGC TACTGGTTTT
 351 ATCGGCAGTC TCAGGACATC TTGGGAAAAC TTCTGACGAC TTACTGTTTA
 401 AAGCTTTAGC ATCTCCCTAT CCTGTCATCC GCTTAGAAGC CGCCTATAGA
 451 CTTGCTAATT TGAAGAACAC TAAAGTCATT GATCATCTAC ATTCTTTCAT
 20 501 TCATAAGCTT CCCGAAGAAA TCCAATGCCT ATCTGCGGCA ATATTCCTAC
 551 GCTTGGAGAC TGAAGAATCT GATGCTTATA TTCGGGATCT CTTAGCTGCC
 601 AAGAAAAGCG CGATTTCGGAG TGCCACAGCT TTGCAGATCG GAGAATACCA
 651 ACAAAAACGC TTTCTTCCGA CACTTAGGAA TTTGCTAACG AGTGCCTCTC
 701 CTC AAGATCA AGAAGCTATT CTTTATGCTT TAGGGAAGCT TAAGGATGGT
 25 751 CAGAGCTACT ACAATATAAA AAAGCAATTG CAGAAGCCTG ATGTGGATGT
 801 CACTTTAGCA GCAGCTCAAG CTTTAATTGC TTTGGGGAAA GAAGAGGACG
 851 CTCTTCCCGT GATAAAAAAG CAAGCACTTG AGGAGCGGCC TCGAGCCCTG
 901 TATGCCCTTAC GGCATCTACC CTCTGAGATA GGGATTCCGA TTGCCCTGCC
 951 GATATTCCTA AAAACFAAGA ACAGCGAAGC CAAGTTGAAT GTAGCTTTAG
 30 1001 CTCTCTTAGA GTTAGGGTGT GACACCCCTA AACTACTGGA ATACATTACC
 1051 GAAAGGCTTG TCCAACCACA TTATAATGAG ACTCTAGCCT TGAGTTTCTC
 1101 TAAGGGGCGT ACTTTACAAA ATTGGAAGCG GGTGAACATC ATAGTCCCTC
 1151 AAGATCCCA GAGAGGGGAA AGGTGCTCT CCACAACCCG AGGTCTTGAA
 1201 GAGCAGATCC TTACGTTTCT CTTCCGCTTA CCTAAAGAAG CTTACCTCCC
 35 1251 CTGTATTTAT AAGCTTTTGG CGAGTCAGAA AACTCAGCTT GCCACTACTG
 1301 CGATTTCTTT TTTAAGTCAC ACCTCACATC AGGAAGCCTT AGATCTACTT
 1351 TTCCAAGCTG CGAAGCTTCC TGGAGAACCT ATCATCCGCG CCTATGCAGA
 1401 TCTTGCTATT TATAATCTCA CCAAAGATCC TGAAAAAAA CGTTCTCTCC
 1451 ATGATTATGC AAAAAAGCTA ATTCAGGAAA CCTTGTTATT TGTGGACACG
 40 1501 GAAAACCAAA GACCCCATCC CAGCATGCC TATCTACGTT ATCAGGTCAC
 1551 CCCAGAAAAGC CGTACGAAGC TCATGTTGGA TATTCTAGAG ACACTAGCCA
 1601 CCTCGAAGTC TTCCGAAGAT ATCCGTTTAT TGATACAAC GATGACGGAA
 1651 GGAGATGCAA AAAATTTCCT AGTCCTTGCA GGCTTACTCA TAAAAATTGT
 1701 GGAGTAA

45 The PSORT algorithm predicts a periplasmic location (0.922).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 25A. The recombinant GST-fusion was used to immunise mice, whose sera were used in a Western blot (Figure 25B) and for FACS analysis (Figure 25C).

50 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6273 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 26

The following *C.pneumoniae* protein (PID 4376735) was expressed <SEQ ID 51; cp6735>:

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1 MTILRNFLTC SALFLALPAA AQVVYLHESD GYNGAINNKS LEPKITCYPE
 51 GTSYIFLDDV RISNVKHDQE DAGVFINRSG NLFFMGNRCN FTFHNLMTGEG
 101 FGAATISNRVG DTTLTLSNFS YLAFTSAPLL PQGGGAIYSL GSVMIENSEE
 151 VTFCGNYSSW SGAAIYTPYL LGSKASRPSV NLSGNRYLVF RDNVSQGYGG
 5 201 AISTHNLTLT TRGPSCFENN HAYHDVNSNG GAIAIAPGGS ISISVKSGDL
 251 IFKGNLTASQD GNTIHNLSIHL QSGAQFKNLR AVSESGVYFY DPISHSESHK
 301 ITDLVINAPE GKETYEGTIS FSGLCDDHE VCAENLTSTI LQDVTLAGGT
 351 LSLSDGVTLQ LHSFKQEQASS TLTMSPGTTL LCSGDARVQN LHILIEDTDN
 401 FVPVRIRAED KDALVSLEKL KVAFEAYWSV YDFPQPKFAP TIPILLELLGP
 10 451 SFDSLLLGET TLERTQVTTE NDAVRGFWSL SWEYPPSLD KDRRITPTKK
 501 TVFLTWNPEI TSTP*

A predicted signal peptide is highlighted.

The cp6735 nucleotide sequence <SEQ ID 52> is:

1 ATGACCATAC TTCGAAATTT TCTTACCTGC TCGGCTTTAT TCCTCGCTCT
 15 51 CCCTGCAGCA GCACAAGTTG TATATCTTCA TGAAAGTGAT GGTATAACG
 101 GTGCTATCAA TAATAAAAGC TTAGAACCTA AAATTACCTG TTATCCAGAA
 151 GGAACCTCTT ACATCTTCTT AGATGACGTG AGGATTCCA ACGTTAAGCA
 201 TGATCAAGAA GATGCTGGGG TTTTATAAAA TCGATCTGGG AATCTTTTTT
 251 TCATGGGCAA CCGTTGCAAC TTCACTTTTC ACAACCTTAT GACCGAGGGT
 20 301 TTTGGCGCTG CCAITTCGAA CCGCGTTGGA GACACCACTC TCACTCTCTC
 351 TAATTTTTCT TACTTAGCGT TCACCTCAGC ACCTCTACTA CCTCAAGGAC
 401 AAGGAGCGAT TTATAGTCTT GGTTCCTGTA TGATCGAAAA TAGTGAGGAA
 451 GTGACTTTCT GTGGGAACCTA CTCTTCGTGG AGTGGAGCTG CGATTATATC
 501 TCCCTACCTT TTAGGTCTTA AGGCGAGTCG TCCTTCAGTA AATCTCAGCG
 25 551 GGAACCGCTA CCTGGTGTTT AGAGACAATG TGAGCCAAGG TTATGGCGGC
 601 GCCATATCTA CCCACAATCT CACACTCACG ACTCGAGGAC CTTCTGTGTTT
 651 TGAAAAATAAT CATGCTTATC ATGACGTGAA TAGTAATGGA GGAGCCATTG
 701 CCAATGCTCC TGGAGGATCG ATCTCTATAT CCGTGAAAAG CGGAGATCTC
 751 ATCTTCAAAG GAAATACAGC ATCACAAGAC GGAAATACAA TACACAACTC
 30 801 CATCCATCTG CAATCTGGAG CACAGTTTAA GAACCTACGT GCTGTTTCAG
 851 AATCCGGAGT TTAITTTCTAT GATCCTATAA GCCATAGCGA GTCGCATAAA
 901 ATTACAGATC TTGTAATCAA TGCTCCTGAA GGAAAGGAAA CTTATGAAGG
 951 AACAAATTAGC TTCTCAGGAC TATGCCTGGA TGATCATGAA GTTTGTGCGG
 1001 AAAATCTTAC TTCCACAATC CTACAAGATG TCACATTAGC AGGAGGAACT
 35 1051 CTCTCTCTAT CGGATGGGGT TACCTTGCAA CTGCATTCTT TTAAGCAGGA
 1101 AGCAAGCTCT ACGCTTACTA TGTCTCCAGG AACCACCTCTG CTCTGCTCAG
 1151 GAGATGCTCG GGTTCAGAAAT CTGCACATCC TGATTGAAGA TACCGACAAC
 1201 TTTGTTCTCG TAAGGATTCTG CGCCGAGGAC AAGGATGCTC TTGTCTCATT
 1251 AGAAAAACTT AAAGTTGCCT TTGAGGCTTA TTGGTCCGTC TATGACTTTC
 40 1301 CTCAATTTAA GGAAGCCTTT ACGATTCTCT TTCTTGAAC TCTAGGCCTT
 1351 TCTTTTGACA GTCTTCTCCT AGGGGAGACC ACTTTGGAGA GAACCCAAGT
 1401 CACAACAGAG AATGACGCCG TTCGAGGTTT CTGGTCCCTA AGCTGGGAAG
 1451 AGTACCCCCC TTCCTCTGGAT AAAGACAGAA GGATCACACC AACTAAGAAA
 1501 ACTGTTTTCC TCACTTGGA TCTTGAGATC ACTTCTACGC CATAA

45 The PSORT algorithm predicts an outer membrane location (0.922).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 26A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 26B).

50 These experiments show that cp6735 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 27

The following *C.pneumoniae* protein (PID 4376784) was expressed <SEQ ID 53; cp6784>:

1 MNRRKARVVV ALFAMTALIS VGCCPWSQAK SRCSDIKYIP VVNRLLEVCV
 55 51 LPEAENVEDL IESSAWVLT PEERFSGELV SICQVKDEHA FYNDLSLLHM
 101 TQAVPSYSAT YDCAVVFGGP LPALRQRLDF LVREWQRGVR FKKIVFLCGE
 151 RGRYQSIEEQ EHFFDSRYNP FPTEENWESG NRVTPSSEEE IAKFVWMQML

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201 LPRAWRDSTS GVRVTFLLAK PEENRVVANR KDTLLLFRRSY QEAFPGRVLF
 251 VSSQPFIGLD ACRVGQFFKG ESYDLAGPGF AQGVLYKHYWA PRICLHTLAE
 301 WLKRTNGCLN ISEGCPG*

A predicted signal peptide is highlighted.

5 The cp6784 nucleotide sequence <SEQ ID 54> is:

1 ATGAATAGAA GAAAAGCAAG ATGGGTAGTG GCATTGTTTCG CAATGACGGC
 51 GCTCATTTCT GTTGGGTGTT GTCCTTGGTC ACAAGCGAAA TCAAGATGTT
 101 CTATTGATAA GTATATTCCT GTAGTCAATC GTTTACTAGA AGTTTGTTGA
 151 CTTCCTGAAG CTGAGAATGT TGAGGATTTA ATCGAGTCCT CGTCGTGCTTG
 201 GGTACTGACT CCTGAAGAAC GTTTTCTGAG AGAGTTAGTC TCTATCTGTC
 251 AGGTTAAAGA TGAGCATGCT TTCTATAACG ATTTGTCTTT ATTACATATG
 301 ACTCAGGCTG TGCCTTCGTA TTCTGCAACG TATGATTGTC CTGTAGTTTT
 351 TGGCGGGCCT TTGCCAGCGC TACGTCAGCG CTTAGATTTT TTGGTGCGAG
 401 AGTGGCAGCG TGGCGTGCGC TTTAAGAAAA TCGTTTTCCT ATGTGGAGAG
 451 CGAGGGCGCT ATCAGTCTAT TGAAGAACAA GAGCATTTCT TTGATCTCTG
 501 GTACAATCCT TTCCCTACTG AAGAGAAGCT GGAATCTGGT AACCGAGTTA
 551 CTCCCTCTTC TGAAGAAGAG ATTGCCAAAT TTGTTTGGAT GCAAAATGCTT
 601 TTACCTAGAG CATGGCGAGA TAGTACTTCA GGAGTCAGAG TGACATTTCT
 651 TCTAGCAAAG CCAGAGGAAA ATCGTGTGGT TGCGAATCGT AAGGACACCT
 701 TACTTTTAT CCCTTCTTAT CAAGAAGCGT TTCCGGGACG CGTGTATTTT
 751 GTAAGTAGTC AACCTTTTAT CGGTTTAGAT GCTTGCAGGG TCGGCGAGTT
 801 TTTCAAAGGG GAAAGCTATG ATCTTGCTGG ACCTGGATTT GCTCAAGGAG
 851 TCTTGAAGTA TCATTGGGCT CCAAGGATTT GTCTACATAC TTAGCGGAA
 901 TGGTTAAAGG AAACGAACGG CTGCTTAAAT ATTTTCAGAGG GTTGTTTTGG
 951 ATGA

The PSORT algorithm predicts a periplasmic location (0.894).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 27A. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 27B). The GST-fusion product was used for FACS analysis (Figure 27C).

30 The cp6784 protein was also identified in the 2D-PAGE experiment (Cpn0498).

These experiments show that cp6784 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 28

The following *C. pneumoniae* protein (PID 4376960) was expressed <SEQ ID 55; cp6960>:

35 1 MNRRWNVLVA TVALALSVAS CDVRSKDKDK DQSLVEYKD NKDTNDIELS
 51 DNQKLSRTFG HLLARQLRKS EDMFFDIAEV AKGLQAEIVC KSAPLTETET
 101 EEKMAEVQKL VFEKRSKENL SLAEKFLKEN SKNAGVVEVQ PSKLQYKIIK
 151 EGAGKAISGR PSALLHYKGS FINGQVFSSS EGNNEPILLP LGQTI PGFAL
 201 GMQGMKEGET RVLYIHPDLA YGTAGQLPPN SLLIFBINLI QASADEVA
 40 251 PQEGNQGE*

A predicted signal peptide is highlighted.

The cp6960 nucleotide sequence <SEQ ID 56> is:

1 ATGAACAGAC GGTGGAATTT AGTTTGTAGCA ACAGTAGCTC TGGCACTCTC
 45 51 CGTCGCTTCT TGTGACGTAC GGTCTAAGGA TAAAGACAAG GATCAGGGGT
 101 CGTTAGTGA ATATAAGAT AACAAAGATA CCAATGACAT AGAATTATCC
 151 GATAATCAAA AGTTATCCAG AACATTGGT CATTATATAG CACGCCAATT
 201 ACGCAAGTCA GAAGATATGT TTTTGTATAT TGCAGAAGTG GCTAAGGGGT
 251 TGCAGCGCGA ATTGGTTTGT AAAAGTGCTC CTTTAACAGA AACAGAGTAT
 301 GAAGAAAAA TGGCTGAAGT ACAGAAGTTG GTTTTGTAAA AAAAATCAAA
 351 AGAAAAATCTT TCATTGGCAG AAAAATTCCT AAAAGAAAA AGCAAGAACG
 50 401 CTGGTGTGTG TGAAGTGCAA CCAAGTAAAT TGCAATACAA AATTATATAA

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451 GAAGGTGCAG GGAAAGCAAT TTCAGGTAAA CCTTCAGCTC TATTGCACTA
 501 CAAGGGTTC TCCATCAATG GCCAAGTATT TAGCAGTCA GAAGGCAACA
 551 ATGAGCCTAT CTGCTTCCT CTAGGCCAAA CAATTCTCTG TTTTGCTTTA
 601 GGTATGCAGG GCATGAAAGA AGGAGAAACT CGAGTTCTCT ACATCCATCC
 651 TGATCTTGCT TACGGAACCG CAGGACAACT TCCTCCAAAC TCTTTATTAA
 701 TTTTGTAAAT TAACTTGATT CAGGCTTCAG CAGATGAAGT TGCTGCTGTA
 751 CCCCAAGAAG GAAATCAAGG TGAATGA

The PSORT algorithm predicts periplasmic space location (0.930).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 28A. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 28B) and for FACS analysis (Figure 28C).

The cp6960 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp6960 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 29

The following *C.pneumoniae* protein (PID 4376968) was expressed <SEQ ID 57; cp6968>:

1 MKFLLYVPLL LVLVSTGCD A KPVSFEPFSG KLSTQRFEPQ HSAREYFSQG
 51 QEFLKKG NFR KALLCPGIIT HHFPRDILRN QAQYLIGVCY FTQDHPDLAD
 101 KAFASYLQLP DAEYSEELFQ MKYALAQ RFA QGKRKRICRL EGFPKLMNAD
 151 EDALRIYDEI LTAFP SKDLG AQALYSKAAL LIVKNDL TEA TKTLKKLTLQ
 201 FPLHILSSEA FVRLSEIYLQ QAKKEPHNLQ YLHFAKLNEE AMKKQHPNHP
 251 LNEVVSANVG AMREHYARGL YATGRFYEKK KKAAANIYY RTAITNYPDT
 301 LLVAKCQKRL DRISKHTS*

A predicted signal peptide is highlighted.

The cp6968 nucleotide sequence <SEQ ID 58> is:

1 ATGAAATTTC TATTATACGT TCCACTTCTT CTGTGTTCTCG TATCTACGGG
 51 GTGCGATGCA AAACCTGTTT CTTTGTAGCC CTTTTCAGGA AAGCTTTCCA
 101 CCCAGCGTTT TGAGCCTCAG CACTCTGCTG AAGAATATTT TTCTCAGGGA
 151 CAGGAATTCT TAAAAAAGG AATTTTCAGA AAAGCTTTTAC TATGCTTTGG
 201 AATCATTTACG CATCAC TTCC CTAGGGACAT CTGCGTAAT CAAGCACAGT
 251 ATCTTTATAGG AGTCTGTTAC TTCACGCAGG ATCACCAGGA TTTAGCAGAC
 301 AAGGCATTTC CATCTTACTT ACAACTTCCT GATGCGGAGT ACTCTGAAGA
 351 GTTGTTCAG ATGAAATATG CGATTGCTCA AAGATTGCTT CAAGGGAAGC
 401 GTAAACGGAT TTGTCGATTA GAGGGCTTCC CAAAACTAAT GAATGCTGAT
 451 GAAGATGCGC TACGCATTTA TGACGAGATT CTAACAGCGT TTCCTAGTAA
 501 AGACTTAGGA GCTCAGGCC TCTATAGTAA AGCTGCGTTA CTTATTGTAA
 551 AAAACGATCT TACAGAAAGC ACCAAAACCT TAAAAAACT CACGTTACAA
 601 TTTCTCTTAC ATATTTTATC TTCAGAGGCC TTTGTACGTT TATCGGAAAT
 651 CTATTTACAG CAAGCTAAGA AAGAGCCTCA CAATCTTCAA TATCTTCATT
 701 TTGCAAAGCT TAATGAAGAG GCAATGAAAA AGCAGCATCC TAACCATCCT
 751 CTGAATGAGG TTGTTTCTGC TAATGTTGGA GCTATGCGGG AACATTATGC
 801 TCGAGGTTTG TATGCCACAG GTCGTTTCTA TGAGAAGAAG AAAAAAGCCG
 851 AGGCTGCGAA TATCTATTAC CGCACTGCGA TTACAACTA CCCAGACACT
 901 TTATTAGTGG CTAAATGTCA AAAGCGTCTA GATAGAATAT CTAAGCATAC
 951 TTCCTAA

The PSORT algorithm predicts an inner membrane location (0.790).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 29A. The recombinant GST-fusion was used to immunise mice, whose sera were used in a Western blot (Figure 29B) and for FACS analysis (Figure 29C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6968 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

5 Example 30

The following *C.pneumoniae* protein (PID 4376998) was expressed <SEQ ID 59; cp6998>:

```

1  MKKLLKSALL SAAFAGSVGS LQALPVGNPS DPSLLIDGTI WEGAAGDPCD
51  PCATWCDATS LRAGFYGDYV FDRILKVDAP KTF5MGAKPT GSAAANYTTA
101 VDRPNPAYNK HLHDAEWFTN AGFIALNIWD RFDVFCFLGA SNGYIRGNST
151 AFNLVGLFGV KGTTVNANEL PNVSL5NGVV ELYTDT5FSW SVGARGALWE
201 CGCATLGAEF QYAQSKPKVE ELNVICNV5Q FSVNKP5GYK GVA5PLPTDA
251 GVATATG5TKS ATINYHEWQV GASLSYRLNS LVPYIGVQWS RATFDADNIR
301 IAQPKLPTAV LNLTAWNPSL LGNATALSTT DSFSDFMQIV SCQINKFKSR
351 KACGVTVGAT LVDADKWSLT AEARLINERA AHVSGQFRF*
```

15 A predicted signal peptide is highlighted.

The cp6998 nucleotide sequence <SEQ ID 60> is:

```

1  ATGAAAAAAC TCTTAAAGTC GGC5GTTATTA TCCGCCGCAT TTGCTGGTTC
51  TGT5TGGCTCC TTACAAGCCT TGCC5TGTAGG GAACCC5TCT GATCCAAGCT
101 TATTAATTGA TGGTACAATA TGGGAAGGTG CTGCAGGAGA TCCT5TGC5AT
151 CCTTGC5GCTA CTTGGT5GCGA CGCTAT5TAGC TTACGT5GCTG GATTTTACGG
201 AGACTATGTT TTCGACCC5TA TCTTAA5AGT AGATGCACCT AAAACAT5TTT
251 CTATGGGAGC CAAGCCTACT GGATCCGCTG CTGCAA5ACTA TACTACTGCC
301 GTAGATAGAC CTAACCCGGC CTACAATAAG CATTTACACG ATGCAGAGTG
351 GTTCACTAAT GCAGGCTTCA TTGCCT5TAAA CATTTGGGAT CGCTTTGATG
401 TTTTCTGTAC TTTAGGAGCT TCTAAT5GGT ACATTAGAGG AAAC5TCTACA
451 GCGTTCAATC TCGTTGGT5T ATT5CGGAGT AAAGGTACTA CTGTAAATGC
501 AAATGAAC5TA CCAAACGTTT CTTTAAGTAA CGGAGT5GTT GAACTTTACA
551 CAGACACCTC TTTCTCT5TGG AGCGTAGGCG CTCGTGGAGC CTTATGGGAA
601 TGCGGT5GTG CAACTTTGGG AGCTGAATTC CAATATGCAC AGTCCA5AACC
651 TAAAGTTGAA GAAC5TTAATG TGATCTGTAA CGTATCGCAA TTCTCTGTAA
701 ACAAA5CCCAA GGGCTATAAA GGCGT5TGCTT TCCCCT5TGCC AACAGACGCT
751 GGCGTAGCAA CAGCTACTGG AACAAAGTCT GCGACCATCA ATTATCATGA
801 ATGGCAAGTA GGAGCCTCTC TATCTTACAG ACTAA5ACTCT TTAGTGCCAT
851 ACATTGGAGT ACAATGGTCT CGAGCA5ACTT TTGATGCTGA TAACATCCGC
901 ATTGCTCAGC CAAA5ACTACC TACAGCTGTT TTAA5ACTTAA CTGCATGGAA
951 CCCTTCT5TTA CTAGGAAATG CCACAGCATT GTCTACTACT GATT5CGTTCT
1001 CAGACTTCAT GCAAAT5GTT TCCTGTCAGA TCAACA5AGTT TAAATCTAGA
1051 AAAGCT5GTG GAGTTACTGT AGGAGCTACT TTAGT5TGATG CTGATAAATG
1101 GTCACTTACT GCAGAAGCTC GTTTAAT5TAA CGAGAGAGCT GCTCACGTAT
1151 CTGGTCAGTT CAGATTCTAA
```

The PSORT algorithm predicts an outer membrane location (0.707).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 30A) and as a his-tag product. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 30B) and for FACS analysis (Figure 30C).

45 The cp6998 protein was also identified in the 2D-PAGE experiment (Cpn0695) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6998 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 31

The following *C.pneumoniae* protein (PID 4377102) was expressed <SEQ ID 61; cp7102>:

```

1  MKHTFTKRVL FFFFLVIPI LLLNLMVVG FFSFAAKANL VQVLHTRATN
51  LSIEFEKKLT IHKLFLDRLA NTLALKSYAS PSAEPYAQAY NEMMALSNTD
101 FSLCLIDPFD GSVRTKNPGD PFIRYLKQHP EMKKKLSAAV GKAFLLTIPG
151 KPLLHYLILV EDVASWDSTT TSGLLVSFYP MSFLQKDLFQ SLHITKGNIC
201 LVNKYGEVLF CAQDSSESSFV FSLDLPLNPQ FQARSPSAIE IEKASGILGG
251 ENLITVSINK KRYLGLVLNK IPIQGTYTLS LVPVSDLIQS ALKVPLNICF
301 FYVLAFLLMW WIFSKINTKL NKPLQELTFC MEAAWRGNHN VRFEPQPYGY
351 EFNELGNIFN CTLLLLLNSI EKADIDVHSG EKLQKELGIL SSLQSALLSP
401 DFPTFPKVTF SSQHLRRRQL SGHFNGWTVQ DGGDTLLGII GLAGDIGLPS
451 YLYALSARSL FLAYASSDVS LQKISKDTAD SFSKTTEGNE AVVAMTFIKY
501 VEKDRSLELL SLSEGAPTMF LQRGESFVRL PLETHQALQP GDRLICLTGG
551 EDILKYFSQL PIEELKDPPL NPLNTENLID SLTMMLNNET EHSADGTLTI
601 LSFS*

```

A predicted signal peptide is highlighted.

The cp7102 nucleotide sequence <SEQ ID 62> is:

```

1  ATGAAACATA CCTTTACCAA GCGTGTCTA TTTTTTTCT TTTTAGTGAT
51  TCCCATTTCC CTACTCCTCA ATCTTATGGT CGTAGGTTTT TTCTCATPTT
101 CTGCCGCTAA AGCAAAATTA GTACAGGTCC TCCATACCCG TGCTACGAAC
151 TTAAGTATAG AATTCGAAAA AAAACTGACG ATACACAAGC TTTTCCTCGA
201 TAGACTTGCC AACACATTAG CCTTAAATC CTATGCATCT CCTTCTGCAG
251 AGCCCTATGC ACAGGCATAC AATGAGATGA TGGCACTCTC CAATACAGAC
301 TTTTCCTTAT GCCTTATAGA TCCCTTTGAT GGATCTGTAA GGACGAAAAA
351 TCCTGGAGAC CCTTTCATTC GCTATCTAAA ACAGCATCCT GAAATGAAGA
401 AAAAGCTATC CGCAGCTGTA GGGAAAGCCT TTTTATTGAC CATTCAGGT
451 AAACCACTTT TACATATCTT TATTCTAGTT GAAGATGTCG CATCTTGGGA
501 TACTACAACG ACTTCAGGAC TGCTTGTAAG TTTCTATCCC ATGCTTTTTT
551 TACCAAAAGA TTTATTTCAA TCCTTACACA TCACCAAAGG AAATATCTGC
601 CTTGTAAATA AGTATGGCGA GGTCTCTTTC TGTGCTCAGG ACAGTGAATC
651 TTCTTTTGTA TTTTCTCTAG ATCTCCCTAA TTTACCGCAA TTCCAAGCAA
701 GAAGCCCTC TGCCATAGAA ATTGAGAAAG CTTCTGGAAT TCTTGGTGGG
751 GAGAACTTAA TCACAGTGAG TATCAACAAG AAACGCTACC TAGGATTGGT
801 ACTGAATAAA ATTCTATACC AAGGGACCTA CACTCTATCT TTAGTTCAG
851 TTTCTGATCT CATCCAATCC GCCTTGAAAG TTCCTCTCAA TATTTGTTTT
901 TTCTATGTAC TTGCTTTCCT CCTCATGTGG TGGATTTTCT CTAAGATCAA
951 CACCAAATTT AACAAGCCTC TTCAAGAACT GACCTTCTGT ATGGAAGCTG
1001 CCTGGCGAGG AAACCATAAC GTGAGGTTTG AACCCAGACC TTACGGTTAT
1051 GAATTCAATG AACTAGGAAA TATTTTCAAT TGCACCTCC TACTCTTATT
1101 GAATTCATTT GAGAAAGCAG ATATCGATTA CCATTGAGG GAAAAATTAC
1151 AAAAAGAATT AGGGATTTTA TCTTCACTAC AAAGTGCCTT ACTAAGTCCG
1201 GATTTCCCTA CGTTCCCTAA AGTTACCTTT AGTTCCCAAC ATCTCCGAG
1251 AAGGCAACTT TCCGGTCATT TTAATGGTTG GACAGTTCAA GATGGTGGCG
1301 ATACCTTTT AGGGATCATA GGGCTCGCTG GCGATATTGG TCTTCTTCC
1351 TATCTCTATG CTTTATCCGC ACGGAGTCTT TTTCTTGCTT ATGCTTCTCT
1401 GGACGTTTCG TTACAAAAAA TCAGCAAGGA TACTGCCGAC AGCTTCTCAA
1451 AAACAACAGA AGGCAATGAG GCTGTAGTTG CTATGACTTT CATTAATAT
1501 GTAGAAAAAG ATCGATCTCT AGAGCTCCTC TCGTTAAGCG AGGGAGCTCC
1551 TACCATGTTT CTACAACGAG GAGAATCTTT CGTACGCTC CCCTTAGAGA
1601 CTCACCAAGC TCTACAGCCT GGAGATCGGT TGATCTGCCT CACTGGAGGA
1651 GAAGACATCC TCAAGTACTT TTCTCAGCTT CCTATTGAAG AGCTCTTAAA
1701 AGATCCTTTA AACCTCTTAA ATACAGAGAA TCTTATTGAT TCTCTAACCA
1751 TGATGTTAAA CAACGAAACC GAACATTCTG CAGATGGAAC TCTGACCATC
1801 CTTTCAATTT CATAA

```

55 The PSORT algorithm predicts an inner membrane location (0.338).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product. The purified GST-fusion product is shown in Figure 31A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot and for FACS analysis (Figure 31B).

These experiments show that cp7102 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 32

The following *C.pneumoniae* protein (PID 4377106) was expressed <SEQ ID 63; cp7106>:

```

5      1 MKDLGTLGGT SSTAKTVSPD GKVIMGRSQI ADGSWHAFMC HTDFSSNNVL
      51 FDLNNTYKTL RENGRLNSI FNLQNMMLQR ASDHEFTFEG RSNIALGAGL
     101 YVNALQNLPS NLAAQYFGIA YKIRPKYRLG VFLDHNFFSSH VPNNFNVSHN
     151 RLWMGAFIGW QSDDALGSSV KVSFGYQKQK ATITREQLN TEAGSGESHF
     201 EGVAAQIEGR YGKSLGGHVR VQPFLGLQFV HITRKEYTEN AVQFPVHYDP
10     251 IDYSTGVVYL GIGSHIALVD SLHVGTMRGM EQNFAAHTDR FSGSIASIGN
     301 FVFEEKLDVTH TRAFEAEMRVN YELPYLQSLN LILRVNQPL QGVMGFSSDL
     351 RYALGF*

```

The cp7106 nucleotide sequence <SEQ ID 64> is:

```

15      1 ATGAAAGATT TGGGGACTCT TGGGGGTACC TCTTCTACAG CAAAAACAGT
      51 GTCCCCAGAT GGTAAAGTGA TCATGGGTAG ATCACAAATT GCTGATGGCA
     101 GTTGGCACGC ATTTATGTGT CATACGGATT TCTCCTCTAA TAATGTACTC
     151 TTTGATCTCG ATAATACGTA TAAACTCTA AGAGAAAATG GCCGTCAGCT
     201 AAATTCCTA TTCAACCTAC AAAATATGAT GTTACAGAGA GCCTCAGATC
     251 ATGAGTTCAC AGAGTTTGGG AGGAGTAACA TCGCTCTTGG TGCCGGGCTT
20     301 TATGTGAATG CCTTGCAGAA TCTCCCTAGC AATTTAGCAG CACAATATTT
     351 TGGAATCGCA TACAAAATAC GTCTTAAATA TCGTTTGGGG GTGTTTGTGG
     401 ACCATAATTT CAGCTCCAC GTTCTTAATA ATTTTAACGT AAGCCACAAT
     451 AGACTCTGGA TGGGAGCCTT TATTGGATGG CAGGATTCTG ATGCTCTAGG
     501 ATCTAGTGTC AAGGTGCTT TCGGATATGG AAAACAAAAA GCCACGATTA
25     551 CAAGAGAGCA ATTAGAGAAT ACAGAAGCCG GGAGTGGGGA GAGCCATTTT
     601 GAAGGGGTCG CTGCTCAGAT AGAAGGGCGG TATGGTAAGA GCCTCGGAGG
     651 ACATGTACAG GTCCAGCCTT TCCTAGGACT GCAGTTTGTC CACATTACAA
     701 GGAAAGAATA TACCGAAAAT GCAGTGCAAT TTCTGTGACA CTATGATCCT
     751 ATAGACTATT CTACAGGTGT AGTGTATTTA GGAATTGGAT CTCATATTGC
30     801 ACTTGTAGAT TCTTTACATG TAGGCACACG CATGGGAATG GAGCAAACT
     851 TTGCAGCCCA TACGGACAGG TTCTCAGGAT CTATAGCGTC TATTGGAAAC
     901 TTTGTGTTTG AAAAGCTTGA TGTGACTCAC ACAAGGGCAT TTGCGGAAAT
     951 GCGTGTCAAC TATGAGCTTC CCTATCTACA GTCTCTGAAT CTTATTCTAC
     1001 GAGTTAATCA ACAGCCTCTA CAAGGGGTTA TGGGATTTTC CAGTGATCTT
35     1051 AGGTATGCCT TAGGATTCTA A

```

The PSORT algorithm predicts a cytoplasmic location (0.224).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product. The purified GST-fusion product is shown in Figure 32A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 32B) and for FACS analysis (Figure 32C).

This protein also showed very good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7106 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 33

The following *C.pneumoniae* protein (PID 4377228) was expressed <SEQ ID 65; cp7228>:

```

      1 MTAVLILTSF PSEBSARSLA RHLITERLAS CVHVFPKGTS TYLWEGKLCE
     51 SEEHHIQIKS IDIRFSEICL AIQEFSGYEV PEVLLFPIEN GDRYLNWLT
    101 ILSYPEKPPL SD*

```


The cp7228 nucleotide sequence <SEQ ID 66> is:

```

      1  ATGACTGCTG TTCTTATTCT TACATCTTTC CCTTCGGAGG AAAGTGCTCG
    51  CTCCTTAGCT AGACATCTGA TTACAGAGCG TCTTGCTTCC TGTGTGCATG
    101  TATTCCTTAA AGGCACATCG ACATATCTAT GGAAGGCAA GCTATGTGAG
    151  TCTGAAGAAC ATCATATACA AATCAAATCG ATAGACATAC GCTTCTCGGA
    201  AATTGTGCTT GCTATTCAGG AGTCTCTGCG CTATGAGGTT CCTGAAGTCT
    251  TACTATTTCC TATTGAAAAT GGGGATCCGA GGTACTTGAA TTGGTTAACG
    301  ATTCCTCAGCT ATCCAGAGAA GCCTCCGCTT TCAGATTAG
  
```

The PSORT algorithm predicts an inner membrane location (0.040).

- 10 The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 33A (his-tag = left-hand arrow, GST = right-hand arrow). The proteins were used to immunise mice, whose sera were used in a Western blot (Figure 33B) and FACS analysis.

These experiments show that cp7228 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

15 Example 34

The following *C.pneumoniae* protein (PID 4377170) was expressed <SEQ ID 67; cp7170>:

```

      1  MNSKMLKHLR LATLSFSMFF GIVSSPAVYA LGAGNPAAPV LPGVNPEQTG
    51  WCAFQLCNSY DLFAALAGSL KFGFYGDYVF SESAHITNVP VITSVTTSQT
    101  GTTPITITST KNVDFDLNNS SISSSCVFAT IALQETSPAA IPLLDIAFTA
    201  RVGGLKQYYR LPLNAYRDFE SNPLNAESEV TDGLIEVQSD YGIVWGLSLQ
    251  KVLWKDGVSF VGVSAHYRHH SSPINYLIVY NKANPEIYFD ATDGNLSYKE
    301  WSASIGISTY LNDYVLPYAS VSIGNPSRKA PSDSFTLELEK QFTNPFKFKIR
      301  KITNFDVRNF CFGTTCCISN NFYYSVEGRW GYQRAINITS GLQF*
  
```

A predicted signal peptide is highlighted.

- 25 The cp7170 nucleotide sequence <SEQ ID 68> is:

```

      1  ATGAATAGCA AGATGCTAAA ACATTACGT TTAGCAACCC TTTCCTTCTC
    51  TATGTTCTTC GGGATTGTAT CTTCTCCCGC AGTATATGCC CTAGGGGCTG
    101  GAAACCCTGC AGCTCCAGTA CTCCCAGGTG TGAATCCTGA GCAAACGGGA
    151  TGGTGTGCCT TCCAACCTTG TAATAGTTAC GATCTTTTTC CTGCTCTTGC
    201  AGGAAGCCTC AAATTGCGT TCTATGGAGA TTATGCTCTC TCAGAAAGTG
    251  CCCATATTAC CAATGTCCCT GTCATTACCT CCGTTACGAC TTCAGGCACA
    301  GGAACAACGC CAACCATTAC CTCTACAAC TAAACCGTAG ACTTTGATCT
    351  TAACAACAGC TCCATCAGCT CGAGCTGTGT TTTTGCAACC ATAGCTCTAC
    401  AGGAACATC CCCAGCTGCC ATTCCCTTT TAGATATAGC CTTCACTGCA
    451  CGTGTGCGAG GACTTAAGCA GTACTACCGC CTCCCTCTCA ATGCTTACAG
    501  AGACTTCACT TCAAATCCTT TAAATGCAGA ATCTGAAGTT ACAGATGGTC
    551  TCATTGAAGT CCAGTCAGAC TATGGAATTG TCTGGGGTCT GAGTTTACAA
    601  AAAGTATTGT GGAAGATGG AGTGCTTTT GTAGGGGTGA GCGCTGACTA
    651  CCGTCACGGT TCCAGTCCCA TCAACTATAT CATCGTTTAC AACAAGGCCA
    701  ACCCGAGAT CTATTTCGAT GCTACTGATG GAAACCTAAG CTATAAAGAA
    751  TGGTCTGCAA GCATCGGCAT CTCTACGTAT CTTAATGACT ATGTGCTTCC
    801  CTATGCATCC GTATCTATAG GAAATACTTC AAGAAAAGCT CCTTCTGATA
    851  GCTTTCACAGA ACTCGAAAAG CAATTACGA ATTTTAAAT TAAAATTCGT
    901  AAAATCACAA ACTTCGACAG AGTAACTTC TGCTTCGGAA CTACCTGCTG
    951  CATCTCAAAT AACTTCTACT ATAGTGTAGA AGGCCGTTGG GGATATCAGC
    1001 GTGCTATCAA CATTACGTCA GGTCTGCAGT TTTAG
  
```

The PSORT algorithm predicts a bacterial outer membrane location (0.936).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product.

The purified GST-fusion product is shown in Figure 34A. The GST-fusion protein was used to

- 50 immunise mice, whose sera were used in a Western blot (34B) and for FACS analysis (34C).

The cp7170 protein was also identified in the 2D-PAGE experiment (Cpn0854).

These experiments show that cp7170 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 35

5 The following *C.pneumoniae* protein (PID 4377072) was expressed <SEQ ID 69; cp7072>:

```

1  MDIKKLFCLF LCSSLIAMSP IYKKTGDYK LTLTGINIID RNGLSETICS
51  KEKLLKKYTKV DFLAPQPYQK VMRMKKNKRG DNVSCLTAYH TNGQIKQYLE
101 CLNNRAYGRY REWHVNGNIK IQAEVIGGIA DLHPSAESGW LFDQTTTFAYN
151 DEGILEAAIV YEKGLLEGSS VYYHTNGNIW KECFYHKGVP QGKFLTYTSS
10  201 GKLLKEQNYQ QGKRHGLSIR YSEDSEEDVL AWEYHHEGRL LKAEYLDPOY
251 HEIYATHEG NGIQAIYGKY AVIETRAFYP GEPIYGVTRF DNSGTQIVQT
301 YNLLQGAHKG BEFFFPYPETG KPKLLLNWHE GILNGIVKTW YPGGTLESCK
351 ELVNNKKSGL LTIYYPEGQI MATEEYDNDL LIKGEYFRPG DRHPYSKIDR
401 GCGTAVFFSS AGTITTKIPY QDGKPLLN*

```

15 A predicted signal peptide is highlighted.

The cp7072 nucleotide sequence <SEQ ID 70> is:

```

1  ATGGATATAA AAAAATCTTT TTGCTTATTT CTATGTTCTT CTCTAATTCG
51  CATGAGTCCC ATTTATGGGA AAACAGGTGA CTATGAGAAA CTCACCCCTA
101  CAGGGATCAA TATCATTTGAT AGAAACGGCC TGTGAGAAAC TATTTGCTCT
20  151 AAAGAGAAGC TAAAGAAATA CACCAAGGTA GACTTTCTTG CTCCCAGCC
201  CTATCAAAAG GTCATGAGGA TGTATAAAAA CAAACGCGGA GATAACGTTT
251  CTTGTTTAAC AGCCTATCAC ACTAACGGGC AAATTAAGCA GTACCTGGAG
301  TGTCTCAATA ATCGTGCTTA TGAAGATAT CGTGAATGGC ACGTCAACGG
351  GAATATCAAA ATCCAAGCTG AGGTTATCGG AGGTATGCG GATCTTCATC
25  401 CCTCAGCAGA GTCTGGCTGG CTATTTGATC AAACACATT TGCCTATAAT
451  GATGAAGGTA TCTTAGAAGC CGCTATCGTC TATGAAAAAG GGCTGCTCGA
501  AGGATCTTCG GTGTATTACC ATACTAATGG GAATATTGG AAAGAGTGTC
551  CCTATCATAA GGGAGTTCTT CAAGGTAAAT TCCTGACATA CACATCTTCG
601  GGGAAACTGC TCAAAGAACA GAATTACCAA CAAGGCAAAA GACACGGTCT
30  651 TTCGATTTCG TACAGCGAAG ATTCCGAAGA AGATGTTTAA GCCTGGGAAG
701  AATATCATGA GGGACGACTC CTAAAAGCAG AGTACTTAGA TCCTCAAAC
751  CACGAAATCT ATGCGACTAT ACACGAAGGG AACGGCATTC AAGCAATCTA
801  CCGCAAGTAT GCCGTTATAG AAACAGGGC ATTTTACCGA GGGGAACCTT
851  ATGGAAGAGT TACCAGATTC GACAACTCCG GAACACAGAT TGTCCAAACG
35  901 TATAACCTTT TGCAAGGCGC GAAGCACGGA GAAGAATTTT TCTTTTATCC
951  TGAGACAGGG AAACCCAAGC TGCTTCTTAA TTGGCATGAA GGAATTTTAA
1001 ATGGGATAGT AAAAATTTGG TATCCCGGAG GAACCTTAGA AAGTTGTAAA
1051 GAACTCGTAA ATAACAAAAA ATCCGGGTTA CTGACCATTT ACTACCCTGA
1101 AGGACAGATC ATGGCGACCG AAGAGTATGA TAATGATCTT CTAATTAAAG
40  1151 GAGAGTACTT CCGCCCTGGA GACCGTCATC CTTACTCTAA AATAGATCGT
1201 GGTTCGTGGA CTGCAGTATT TTTCTCGTCG GCGGGAAC TA TACTAAAAA
1251 AATCCCTTAT CAGGACGGCA AACCTTTGCT CAACTAG

```

The PSORT algorithm predicts a periplasmic location (0.688).

45 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 35A) and as a GST-fusion product (Figure 35B). The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 35C) and for FACS analysis.

These experiments show that cp7072 is a useful immunogen. These properties are not evident from the sequence alone.

Example 36

50 The following *C.pneumoniae* protein (PID 4376879) was expressed <SEQ ID 71; cp6879>:

```

1  MATPAQKSPT FQDPSFVREL GSNHPVFSPL TLEERGEMAI ARVQQCGWNH
51  TIVKVSILIL ALLTILGGGL LVGLLPVAVPM FIGTGLIALG AVIFALALIL
101 CLYDSQGLPE ELPPVPEPQQ IQIEDLRNET REVLEGTLLLE VLLKDRDAKD
151 PAVPQVVVDC EKRLGMLDRK LRREEEILYR STAHLKDEER YEFLLELLEM
201 RSLVADRLEF NRRSYERFVQ GIMTVRSEEG EKEISRLQDL ISLQQQTVDQ
251 LRSRIDDEQK RCWTALQRIN QSQKDIQRAH DREASQRACE GTEMDCAERQ
301 QLEKDLRRQL KSMQEWIEMR GTIHQQEKAW RKQNAKLERL QEDLRLTGIA
351 FDEQSLFYRE YKEKYSQKL DMQKILQEVN AEKSEKACLE SLVHDYKQQL
401 BQKDANLKA AAVWEEELGK QQQEDYEQTO EIRRLSTFIL EYQDSLREAE
451 KVEKDFQELQ QRYSLRQEEK QVKEKILEES MNHFADLFK AQKENMAYKK
501 KLADLEGAAA PTEIGEDDDW VLTDSASLSQ KKIRELVEEN QELLKALAFK
551 SNETQLVAD AVEAEKEISK LREHIKEQKB GLRALDKMHA QAIKDCAAQ
601 RKCCDLESLL SPVREDAGMR FELEVELQRL QEENALRAE VERLEQEQFO
651 G*

```

The cp6879 nucleotide sequence <SEQ ID 72> is:

```

1  ATGGCAACAC CCGCTCAAAA ATCCCCTACA TTTCAGATC CTAGTFTTGT
51  AAGAGAGCTA GGCAGTAACC ACCCTGTCTT TTCCCGCTA ACGCTTGAGG
101 AAAGAGGGGA GATGGCAATA GCTCGAGTCC AGCAGTGTGG ATGGAATCAT
151 ACAATTTGTTA AGGTAAGTCT TATTATCTTT GCTCTTCTTA CTATTTTAGG
201 GGGAGGATTA CTCGTAGGAT TGCTGCCAGC AGTTCCTATG TTTATTGGAA
251 CAGGTCTGAT TGCTTTGGGA GCCGTATATAT TTGCTTTGGC TTTGATTTTA
301 TGTCTTTATG ATTCTCAGGG CCTTCCTGAG GAACTCCCTC CGGTTCCTGA
351 ACCACACAA ATTACAGATTG AAGATTTAAG AAACGAGACC AGAGAAGTTC
401 TTGAAGGGAC TCTTTTAGAG GTTCTCTTAA AGGATAGAGA CGCTAAGGAC
451 CCTGCGGTGC CCCAGGTGGT TGCTAGACTGT GAAAAGCGTC TTGGAATGTT
501 GGATCGTAAG CTGCGACGTG AAGAGGAGAT TCTGTATCGC TCGACGGCCC
551 ATCTTAAAGA CGAGGAAAGG TATGAGTTCT TGCTGGAGCT CTTGGAATG
601 CGTAGTCTGG TTGCCGATCG GCTAGAATT T AACCGTAGAA GTTATGAGCG
651 ATTTGTTCAA GGAATTATGA CAGTTAGATC AGAGGAGGGG GAAAAAGAGA
701 TTTCTCGTCT ACAAGATCTA ATCAGTTTGC AGCAGCAGAC GGTGCAAGAT
751 TTAAGGAGTC GGATCGATGA CGAGCAGAAG AGATGCTGGA CGGCTTTACA
801 ACGTATTAAC CAATCTCAGA AGGATATACA ACGGGCTCAT GATCGCGAGG
851 CTTCCGACGC TGCCCTGTGAG GGCACAGAGA TGGATTGTGC AGAACGCCAG
901 CAACGTGAGA AGGATTTAAG GAGACAGCTG AAATCTATGC AGGAGTGGAT
951 TGAGATGAGG GGCACAATCC ATCAACAAGA GAAGGCTTGG CGTAAGCAGA
1001 ATGCCAAATT AGAAAGATTA CAAGAGGATC TGAGACTTAC TGGGATTTGT
1051 TTTGACGAAC AATCTCTGTT CTATCGCGAA TATAAAGAGA AATATCTGAG
1101 TCAGAACTA GATATGCAA AGATTTTACA GGAAGTCAAC GCAGAGAAAA
1151 GTGAGAAGGC TTGCTTAGAG AGTCTGGTCC ATGACTATGA GAAGCAGCTC
1201 GAACAAAAAG ATGCTAATCT GAAGAAAGCA GCAGCTGTTT GGAAGAAGA
1251 ATTAGGGAAG CAGCAACAGG AAGACTACGA ACAAAACCAA GAAATTAGAC
1301 GTCTGAGTAC ATTCAATCTT GAGTACCAGG ACAGTCTGCG TGAGGCAGAA
1351 AAAGTTGAGA AAGATTTCCTA AGAGCTACAA CAAAGGTATA GCCGTCTTCA
1401 AGAGGAGAAA CAGGTAAGG AAAAAATCTT AGAAGAAAGT ATGAATCATT
1451 TTGCCGATCT CTTTGAGAAG GCTCAAAAGG AAAACATGGC CTACAAGAAG
1501 AAGTTAGCGG ATTTAGAGGG TGCCGCTGCT CCTACTGAGA TCGGTGAGGA
1551 CGATGACTGG GACTCACAG ATTCTGCTTC TCTCAGCCAG AAGAAGATCC
1601 GCGAACTCGT GGAAGAGAAT CAAGAACTCC TGAAAGCACT TGCATTFAAA
1651 TCTAACGAAT TGACTCAACT GGTGCCGAT GCTGTAGAAG CTGAAAAAGA
1701 AATCAGCAAG CTTGAGAAC ACATAGAAGA GCAGAAAGAA GGATTACGAG
1751 CTCTTGATAA GATGCATGCA CAAGCGATCA AAGATTGCGA AGCTGCTCAG
1801 AGAAAAATGCT GTGACCTTGA GAGCCCTCTC TCTCCTGTTT GAGAAGATGC
1851 TGGAATGAGA TTTGAGCTAG AGGTCGAGCT TCAAAGATTG CAAGAAGAAA
1901 ATGCACAGCT TAGAGCGGAG GTTGAAAGAC TAGAGCAAGA GCAATTTCAA
1951 GGATAA

```

The PSORT algorithm predicts an inner membrane location (0.646).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product. The purified GST-fusion product is shown in Figure 36A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 36B) and for FACS analysis.

These experiments show that cp6879 is useful immunogen. These properties are not evident from the sequence alone.

Example 37

The following *C.pneumoniae* protein (PID 4376767) was expressed <SEQ ID 73; cp6767>:

```

1  MIKQIGRFFR AFIFIMPLSL TSCESKIDRN RIWIVGTNAT YPPFEYVDAQ
5  51  GEVVGFDIDL AKAISEKLGK QLEVREPAFD ALILNLKKHR IDAILAGMSI
101 TFSRQKEIAL LPYYGDEVQE LMVVSQRSLE TPVLPLTQYS SVAVQTGTFQ
151 EHYLLSQPGI CVRSFDSLLE VIMEVRYGKS PVAVLEPSVG RVVLKDFPNL
201 VATRLELPPE CWVLGCGLGV AKDRPEEIQT IQQAITDLKS EGVISLTKK
251 WQLSEVAYE*

```

The cp6767 nucleotide sequence <SEQ ID 74> is:

```

10 1  ATGATAAAAC AAATAGGCCG TTTTTTTAGA GCATTTATTT TTATAATGCC
51  TTTATCTTTA ACAAGTTGTG AGTCTAAAAT CGATCGAAAT CGCATCTGGA
101 TTGTAGGTAC GAATGCTACA TATCCTCCTT TTGAGTATGT GGATGCTCAG
151 GGGGAAGTTG TAGGTTTCGA TATAGATTTC GCAAAGGCAA TTAGTGAAAA
201 ACTTGGCAAG CAATTGGAAG TTAGAGAATT CGCTTTCGAT GCTTTAATTT
15 251 TAAATTTAAA AAAACATCGT ATCGATGCAA TTTTAGCAGG AATGTCCATT
301 ACTCCTTCGC GTCAGAAGGA AATCGCCTG CTTCCTTATT ATGGCGATGA
351 GGTTCAGAG CTGATGGTGG TTTCTAAGCG GTCTTTAGAG ACCCCTGTGC
401 TCCCCTAAC ACAGTATTCT TCTGTTGCTG TTCAGACAGG AACGTTTCAG
451 GAGCATTTATC TTTTATCTCA GCCCGGAATT TGTGTCCGTT CTTTGATAG
20 501 CACCTTGGAG GTGATTATGG AAGTTCGTTA TGGGAAATCT CCGGTTGCCG
551 TTCTAGAACC CTCGGTAGGA CGTGTCTGTC TTAAAGACTT CCCTAATCTT
601 GTTGCAACAA GATTAGAGCT CCCTCCTGAA TGTGGGTGTG TGGGCTGTGG
651 TCTCGGCGTA GCTAAAGATC GTCCTGAAGA AATACAAACG ATTCAACAAG
701 CGATTACAGA TTAAAGAGC GAAGGGGTGA TTCAATCTTT AACCAAGAAA
25 751 TGGCAACTTT CTGAAGTTGC TTACGAATAG

```

The PSORT algorithm predicts an inner membrane location (0.083).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product. The purified his-tag product is shown in Figure 37A. The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 37B) and for FACS analysis (Figure 37C). The GST-fusion was also used in a Western blot (Figure 37D).

The cp6767 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6767 is a useful immunogen. These properties are not evident from the sequence alone.

Example 38

The following *C.pneumoniae* protein (PID 4376717) was expressed <SEQ ID 75; cp6717>:

```

1  MMSRLRFRLA ALGIFFILLV PNSVSAKTIV ASDKREKVGVL VYDNSVEAFQ
51  QILDCTIDHAN FYVELCPCMT GGRTLKEMVD HLEARMDLVP ELCSYIIIQF
101 TPTDAEDQKL LKALKERHPN RFFYVFTGCP PSTSILAPNV IEMHIKLSII
40 151 DGKYCILGGT NFEFMCTPG DEVPEKVDNP RLFVSGVRRP LAFRDQDIML
201 RSTAFGLQLR EBYHKQFAMW DYYAHMWF I DNPEQFAGAC PPLTLEQAE
251 TVFPGFDKHE DLVLVDSSKI RIVLGGPHDK QPNPVTQEYL KLIQGARSSV
301 KLAHMYFIPK DELLNALVDV SHNHGVHLSL ITNGCHELSP AITGPHYAWN
351 RINYFALLYG KRYPLWKKWF CEKLPYERV SIYEFATWET QLHKCMIID
45 401 DEIFVIGSYN FGKKSDAFDY ESIVVIESPE VAAKANKVFN KDIGLSIPVS
451 HGDIFSWYFH SVHHTLGHLLQ LTYMPA*

```

A predicted signal peptide is highlighted.

The cp6717 nucleotide sequence <SEQ ID 76> is:

-78-

```

1  ATGATGAGTC GGTTCGCTTT TCGCTTGGCA GCTCTTGGAA TATTTTITAT
51  TTTGCTGGTT CCTAATTCCTG TTTCAGCAAA GACAATCGTA GCTTCAGACA
101 AGGAGAAGGT TGGAGTTCCTT GTTTATGACA ATAGTGTAGA GGCCTTTCAA
151 CAGATATTGG ATTGCATAGA TCATGCAAAT TTTTATGTAG AACTGTGTCC
5    201 CTGCATGACA GGAGGCCGAA CGCTTAAAGA GATGGTAGAT CACCTCGAGG
251 CTCGTATGGA TCTGGTTCCA GAGCTCTGTA GCTATATCAT TATCCAACCC
301 ACGTTTACCG ATGCTGAAGA CAAAAATTA CTCAAAGCTC TCAAAGAACG
351 TCATCCCAAC CGGTTTTCTT ACGTTTITAC AGGGTGCCCA CCCTCAACAA
401 GCATCCTCGC TCCTAATGTC ATTGAAATGC ATATCAAAC TTTCTATCAT
10   451 GATGGGAAAT ATTGTATTTT AGGTGGTACC AATTTTGAAG AGTTTATGTG
501 CACTCCAGGG GATGAGGTTT CTGAGAAAGT GGATAACCCA CGTTTATTTG
551 TCAGTGGAGT GCGTCGGCCC CTAGCATTTT GTGATCAGGA TATCATGTTG
601 CGTTCTACAG CATTCGGTTT GCAGCTCAGA GAAGAATATC ATAAGCAATT
15   651 GACTATGTGG GACTACTATG CACATCATAT GTGGTTCATT GATAATCCTG
701 AACAGTTTGC AGGCGCCTGT CCTCCACTGA CTTTAGAACA AGCCGAGGAG
751 ACAGTATTTT CTGGATTGTA CAAACATGAA GATCTTGTTT TTGTCGACTC
801 TTCCAAGATC AGGATAGTTT TAGGTGGTCC CCACGATAAG CAACCCAATC
851 CTGTGACTCA AGAATATTTG AAACCTATCC AGGGAGCTAG ATCTTCTGTG
901 AAGCTTGCTC ACATGTATTT CATCCCTAAG GACGAGCTTT TAAATGCTCT
20   951 TGTCGACGTT TCTCATAATC ACGGTGTTCA TCTGAGTTTA ATTACGAACG
1001 GCTGTCATGA ATTAAGTCC T GCAATTACAG GACCCATATG TTGGGGAAAC
1051 CGTATTAACT ATTTTCGCCTT GCTCTATGGG AAACGGTATC CTCTTTGGAA
1101 AAAATGGTTT TGCGAAAAGC TAAAACTTTA TGAGCGGGTT TCTATTATG
1151 AGTTTGCTAT TTGGGAAACG CAGTTGCACA AGAAGTGTAT GATTATCGAT
25   1201 GATGAAATTT TTGTGATCGG AAGTTATAAT TTTGGAAAGA AAAGTGATGC
1251 CTTTGATTAC GAAAGTATTG TAGTTATCGA ATCTCCAGAA GTCGCTGCAA
1301 AAGCTAACAA AGTCTTCAAT AAAGATATCG GATTGTGAT TCCTGTAAGT
1351 CATGGCGACA TTTTCTCTTG GTATTTCAT TCCGTACACC ACACTTGGG
1401 ACATTTGCAG CTGACCTATA TGCCAGCCTA G

```

The PSORT algorithm predicts a periplasmic location (0.939).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 38A), as a his-tagged protein, and as a GST/his fusion product. The proteins were used to immunise mice, whose sera were used in a Western blot (Figure 38B) and for FACS analysis.

These experiments show that cp6717 is a useful immunogen. These properties are not evident from the sequence alone.

Example 39

The following *C.pneumoniae* protein (PID 4376577) was expressed <SEQ ID 77; cp6577>:

```

1  MKRLLPSTFL LVLGSTSAAH ANLGYVNLKR CLLEESDLGRK ETEELRAMKQ
51  QFVKNAEKIE BELTSYNNKL QDEDYMESLS DSASEELRKK FEDLSGEYNA
40  101 YQSQQYQSIN QSNVKRIQKL IQEVKIAAES VRSKEKLEAI LNEEAVLAIA
151 PGTDRTTEII AILNESFKKQ N*

```

A predicted signal peptide is highlighted.

The cp6577 nucleotide sequence <SEQ ID 78> is:

```

1  ATGAAAAAAT TATTATTTTC TACATTTCCTT CTGTGTTTAG GATCAACAAG
45  51  CGCAGCTCAT GCAAATTTAG GCTATGTTAA TTTAAAGCGA TGTCTTGAAG
101  AATCCGATCT AGGTAAAAAG GAAACTGAAG AATTGGAAGC TATGAAACAG
151  CAGTTTGTA AAAAAAGCTGA GAAAAAGTAA GAAGAACTCA CTTCTATTTA
201  TAATAAGTTG CAAGATGAAG ATTACATGGA AAGCCTATCG GATTCTGCCT
251  TCGAAGAGTT GCGAAAAGAA TTCGAAGATC TTTGAGGAGA GTACAATGCG
50  301  TACCAGTCTC AGTACTATCA ATCTATCAAT CAAAGTAATG TAAAACGCAT
351  TCAAAAACCTC ATTCAAGAAG TAAAAATAGC TGCAGAATCA GTGCGGTCCA
401  AAGAAAAACTC AGAAGCTATC CTTAATGAAG AAGCTGTCTT AGCAATAGCA
451  CCTGGGACTG ATAAAACAAC CGAAATTATT GCTATTCTTA ACGAATCTTT
501  CAAAAAACAA AACTAG

```

The PSORT algorithm predicts a periplasmic space location (0.932).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 39A) and as a GST-fusion product (Figure 39B). The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 39C) and for FACS analysis.

The cp6577 protein was also identified in the 2D-PAGE experiment.

- 5 These experiments show that cp6577 is a useful immunogen. These properties are not evident from the sequence alone.

Example 40

The following *C.pneumoniae* protein (PID 4376446) was expressed <SEQ ID 79; cp6446>:

```

1  MKQPMSLIFS SVCLGLGLGS LSSCNQKPSW NYHNTSTSEE FFVHGKNSVS
51  QLPHPYSAFR TTQIFSEEHN DPYVVAKTDE ESRKIWREIH KNLKIKGSYI
101 PISTYGSLMH PKSAALTLKT YRPHPIWING YERSFNIDTG KYLKNGSRRR
151 TSHDGPKNRA VLNLKSSGR RCNAIGLEMT EEDFVIARRR EGVYSLYPVE
201 VCSYPQGNPF VIAYAWIADE SACSKEVLPV KGYYSLVWES VSSSDSLNAF
251 GDSFAEDYLR STFLANGTSI LCVHESYKKV PPQP*
```

- 15 A predicted signal peptide is highlighted.

The cp6446 nucleotide sequence <SEQ ID 80> is:

```

1  ATGAAACAGC CCATGTCTCT TATCTTTTCA AGTGTATGTT TAGGATTAGG
51  TCTTGGATCT CTTCCTCCTT GTAATCAAAA GCCCTCTTGG AATTATCACA
101  ACACTTCAAC GAGCGAAGAA TTCCTTGTTC ATGGAAATAA GAGTGTTCG
201  CAACTGCCTC ATTATCCTTC TGCATTTTCG ACGACTCAAA TCCTTCTCTGA
251  AGAGCACAAT GATCCTTATG TCGTAGCTAA GACTGATGAA GAGTCTCGTA
301  AAAATTGGAG AGAAATCCAT AAAAATCTCA AAATCAAAGG TTCTTACATT
351  CCCATATCGA CTTATGGAAG TCTGATGCAC CCAAATCAG CAGCTCTTAC
401  ATTAAAAAACG TATCGTCCAC ATCCTATTTG GATAAATGGA TACGAGCGTT
451  CTTTTAATAT AGACACAGGA AAGTACTTAA AAAACGGAAG TCGCCGTAGA
501  ACTTCTCAGC ATGGTCCGAA AAATCGAGCT GTACTGAATC TCATTAAATC
551  TTCCGGACGA CGCTGTAATG CTATAGGCCT TGAGATGACA GAAGAAGACT
601  TTGTAATAGC TAGAAGGCCA GAAGGTGTTT ATAGCCTGTA TCCCGTTGAA
651  GTGTGCTCGT ATCCTCAGGG GAATCCTTTT GTCATGCTT ATGCCTGGAT
701  TGCAGATGAG AGTGTCTGCT CAAAAGAGGT CCTACCTGTA AAAGGGTACT
751  ATTCTTTAGT CTGGGAAAGC GTTCTTTCCT CTGATCTCTT GAATGCTTTT
801  GGAGATTCCT TTGCAGAGGA CTACCTCAGA AGCACGTTTT TAGCAAACGG
851  AACTTCTATA CTCGTGTGTC ATGAAAGCTA TAAGAAAGTT CCTCCTCAGC
CCTAA
```

- 35 The PSORT algorithm predicts an inner membrane location (0.177).

The protein was expressed in *E.coli* and purified as a his-tag product and a GST-fusion product. The GST-fusion product is shown in Figure 40A. The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 40B) and for FACS analysis.

- 40 These experiments show that cp6446 is a useful immunogen. These properties are not evident from the sequence alone.

Example 41

The following *C.pneumoniae* protein (PID 4377108) was expressed <SEQ ID 81; cp7108>:

```

1  MSKKIKVLGH LTLCTLFRGV LCAAALSNIG YASTSQESPY QKSIEDWKGY
51  TFTDLELLSK EGWSEAHAVS GNGSRIVGAS GAGQGSVTAV IWESHLIKHL
101  GTLGGRASSA EGISKDGEV VGWSDTREGY THAFVFDGRD MKDLGTLGAT
151  YSVARGVSGD GSIIVGVSAT ARGEDYGWQV GVKWERGKIK QLKLLPQGLW
```

-80-

201 SEANAISEDG TVIVGRGEIS RNHIVAVKWN KNAVYSLGTL GGSVASAEAI
 251 SANGKVIVGW STTNNGETHA FMHKDETMHD LGTLGGGFSV ATGVSADGRA
 301 IVGFSAVKTG EIHAFYYAEG EMEDLTTLGG EEARVFDISS EGNDIIGSIK
 351 TDAGAERAYL FHIHK*

5 A predicted signal peptide is highlighted.

The cp7108 nucleotide sequence <SEQ ID 82> is:

1 ATGAGTAAGA AGATAAAGGT TCTAGGTCAT TTGACGCTCT GCACTCTGTT
 51 TAGAGGAGTG CTGTGTGCAG CGGCCCTTTC CAACATAGGA TATGCGAGTA
 101 CTTCTCAGGA ATCACCATAT CAGAAGTCTA TAGAAGACTG GAAAGGGTAT
 10 AACTTTACAG ATCTTGAGTT ACTGAGTAAG GAAGGGTGGT CTGAAGCTCA
 201 TGCAGTTTCT GGAAATGGCA GTAGAATTGT AGGAGCTTCG GGAGCTGGCC
 251 AAGGTAGTGT GACTGCTGTC ATATGGGAAA GTCACCTGAT AAAACATCTC
 301 GGCACTTTAG GTGGCGAGGC TTCATCTGCA GAGGGAATTT CAAAGGATGG
 351 AGAGGTGGTC GTTGGGTGGT CAGATACTAG AGAGGGATAT ACTCATGCCT
 15 401 TTGCTTTCGA CGGTAGAGAT ATGAAAGATC TCGGTACTCT AGGAGCTACC
 451 TATTCCTAG CAAGGGGTGT TTCTGGAGAT GGTAGTATCA TCGTAGGAGT
 501 CTCTGCAACT GCTCGTGGAG AGGATTACGG ATGGCAAGTT GGTGTCAAGT
 551 GGGAAAAGG GAAAATCAAA CAATTGAAGT TGTTCCTCA AGGTCTCTGG
 601 TCTGAGGCGA ATGCAATCTC TGAGGATGGT ACGGTGATTG TCGGGAGAGG
 20 651 TAGGAATCTCT CGCAATCACA TCGTTGCTGT AAAATGGAAT AAAAATGCTG
 701 TGTATAGTTT GGGGACTCTC GGAGGTAGTG TCGCTTCAGC AGAGGCTATA
 751 TCGGCAAATG GGAAAGTAAT TGTAGGATGG TCCACGACTA ATAATGGTGA
 801 GACTCATGCC TTTATGCACA AAGATGAGAC AATGCACGAT CTCGGCACTC
 851 TAGGAGGAGG TTTTCTGTG CCAACTGGAG TTCTGTCTGA TGGGAGAGCC
 25 901 ATCGTAGGAT TTTCAGCAGT GAAGACCGGA GAAATTCATG CTTTTTACTA
 951 TGCAGAAGGA GAAATGGAGG ATTTAACAAC TTGGGAGGG GAAGAAGCTC
 1001 GAGTGTTCGA CATATCTAGC GAAGGAAACG ATATCATTTG CTCTATAAAA
 1051 ACTGACGCTG GAGCTGAACG CGCCTATCTG TTCCATATAC ATAAATAA

The PSORT algorithm predicts an outer membrane location (0.921).

30 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 41A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 41B) and for FACS analysis (Figure 41C). A his-tagged protein was also expressed.

The cp7108 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp7108 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 42

The following *C.pneumoniae* protein (PID 4377287) was expressed <SEQ ID 83; cp7287>:

1 MVAKRTVRSY RSSFHSVIV AILSAGIAFE AHSLHSSELD LGVFNKQFRE
 51 HSAHVEEAQT SVLKGSDFVN PSQKESEKVL YTVVPLTQGS SGESLDLADA
 40 101 NFLEHFQHLF KETTTFGIDQ KLVWSDLDTR NPSQPTQEPD TSNAVSEKIS
 151 SDTKENRKDL ETEDPSKKSG LKEVSSDLPK SPETAVAAIS EDLEISENIS
 201 ARDPLQGLAF FYKNTSSQSI SEKDSSFQGI IFSGSGANSQ LGFENLKAPK
 251 SGAAVYSRDR IVFENLVKGL SFISCESLED GSAAGVNIVV THCGDVTITD
 301 CATGLDLEAL RLVKDFSRGG AVFTARNHEV QNNLAGGILS VVGKNGAIVV
 45 351 EKNSAEKSNQ GAFACGSFVY SNNENTALWK ENQALSGGAI SSASDIDIQG
 401 NCSAIEPSGN QSLIALGEHI GLTDFVGGGA LAAQGTTLTR NNAVVCVKN
 451 TSKTHGAIL AGTVDLNETI SEVAFKQNTA ALTGGALSAN DKVIIANNFG
 501 EILFEQNEVR NHGGAICYGC RSNPKLEQKD SGENINIIGN SGAITFLKNK
 551 ASVLEVMTQA EDYAGGSAW GHNVLLDSNS GNIQFIGNIG GSTFWIGEVV
 50 601 GGGAILSTDR VTISNNSGDV VFKGNKGQCL AQKYVAPQET APVESDASST
 651 NKDEKSLNAC SHGDHYPPKT VEEVPPSLI EEHPVVSSTD IRGGGAILAQ
 701 HIFITDNTGN LRFSGNLGGG EESSTVGDLA IVGGGALLST NEVNVCSNQN
 751 VVFSNDVTSN GCDSGGAILA KKVDISANHS VEFVSNSSGK FGGAVCALNE
 801 SVNITDNGSA VSFSKNRTRL GGAGVAAPQG SVTICGNQGN IAPKENFVFG

-81-

851 SENQRSGGGA IANSSSVNIQ DNAGDILFVS NSTGSYGGAI FVGSVLVASEG
 901 SNPRTLTITG NSGDILFAKN STQTAASLSE KDSFSGGAIY TQNLKIVKNA
 951 GNVSPFYGNRA PSGAGVQIAD GGTVCLEAFG GDILFEGNIN FDGSFNAILHL
 1001 CGNDSKIVEL SAVQDKNIIF QDAITYEENT IRGLPDKDVS PLSAPSLIFN
 1051 SKPQDDSAQH HEGTIRFSRG VSKIPQIAAI QEGTLALSON AELWLALGLKQ
 1101 ETGSSIVLSA GSILRIFDSQ VDSSAPLPTE NKEETLVSAG VQINMSSPTP
 1151 NKDKAVDTPV LADIISITVD LSSFVPEQDG TLPLPPEIII PKGTXLHNSA
 1201 IDLKIIDPTN VGYENHALLS SHKDIPLISL KTAEGMTGTP TADASLSNIK
 1251 IDVSLPSITP ATYGHGTGWS ESKMEDGRLV VGWQPTGYKL NPEKQALVL
 1301 NNLWSHYTDL RALKQEIFAH HTIAQRMELD FSTNVWGSGL GVVEDCQNIQ
 1351 EFDGFKHHLT GYALGLDTQL VEDFLIGGCF SQFFGKTESQ SYKAKNDVKS
 1401 YMGAAYAGIL AGPWLIKGA FVYGNINNDLT TDYGTGLIST GSWIGKGFIA
 1451 GTSIDYRYIV NPPRFISATV STVVPFVEAE YVRIDLPEIS BQGKEVRTFQ
 1501 KTRFENVAIP PGFALEHAYS RGSRAEVNSV QLAVYFDVYR KGPVSLITLK
 1551 DAAYSWSYSG VDIPCKAWKA RLSNNTWNS YLSTYLAIFY EWREDLIAYD
 1601 FNGGIRIIF*

A predicted signal peptide is highlighted.

The cp7287 nucleotide sequence <SEQ ID 84> is:

1 ATGGTAGCGA AAAAAACAGT ACGATCTTAT AGGCTCTTCAT TTTCTCATTC
 51 CGTAATAGTA GCAATATTGT CAGCAGGCAT TGCTTTTGAA GCACATTCCT
 101 TACACAGCTC AGAAGTAGAT TTAGGTGTAT TCAATAAACA GTTTGAGGAA
 151 CATTCTGCTC ATGTTGAAGA GGCTCAAACA TCTGTTTAA AGGGATCAGA
 201 TCCTGTAAAT CCCTCTCAGA AAGAATCCGA GAAGGTTTG TACACTCAAG
 251 TGCTCTCTAC CCAAGGAAGC TCTGGAGAGA GTTTGATCT CGCCGATGCT
 301 AATTCTTAG AGCATTTTCA GCATCTTTT GAAGAGACTA CAGTATTTGG
 351 TATCGATCAA AAGCTGGTTT GGTCAGATT AGATACTAGG AATTTTCCC
 401 AATCCACTCA AGAAGCTGAT ACAAGTAATG CTGTAAGTGA GAAATCTCC
 451 TCAGATACCA AAGAGATAG AAAAGACCTA GAGACTGAAG ATCCTTCAA
 501 AAAAAAGTGGC CTTAAGAAG TTTTATCAGA TCTCCCTAAA AGTCCCTGAAA
 551 CTGCAGTAGC AGCTATTTCT GAAGATCTTG AAATCTCAGA AAACATTTCA
 601 GCAAGAGATC CTCTTCAGGG TTTAGCATTT TTTTATAAAA ATACATCTTC
 651 TCAGTCTATC TCTGAAAAGG ATTCTTCATT TCAAGGAATT ATCTTTTCTG
 701 CTTTCAGGAGC TAATTCAGGG CTAGGTTTGG AAAATCTTAA GCGCGCGAAA
 751 TCTGGGGCTG CAGTTTATTC TGATCGAGAT ATTGTTTGG AAAATCTTGT
 801 TAAAGGATTG AGTTTATAT CTGTGTAATC TTTAGAGAT GGCTCTGCCG
 851 CAGGTGTAAG CATTGTTGTG ACCCATTTGT GTGATGTAAC TCTCACATGAT
 901 TGTGCCACTG GTTTAGACCT TGAAGCTTTA CGTCTGGTTA AAGATTTTTC
 951 TCGTGGAGGA GCTGTPTTCA CTGCTCGCAA CCATGAAGTG CAAAATAACC
 1001 TTGCAGGTGG AATCTATATC GTTGTAGGCA ATAAAGGAGC TATTGTGTA
 1051 GAGAAAAATA GTGCTGAGAA GTCCAAATGGA GGAGCTTTTG CTTGCGGAAG
 1101 TTTTGTPTTAC AGTAACAACG AAAACACCGC CTGTGAGGAA GAAATCAAG
 1151 CATTATCAGG AGGAGCCATA TCCTCAGCAA GTGATATTGA TATTCAAGGG
 1201 AACTGTAGCG CTATTGAATT TTCAGGAAAC CAGTCTCTAA TTGCTCTTGG
 1251 AGAGCATATA GGGCTTACAG ATTTTGTAGG TGGAGGAGCT TTAGCTGCTC
 1301 AAGGGACGCT TACCTTAAGA AATAATGCAG TAGTGCAATG TGTAAAAAC
 1351 ACTTCTAAAA CACATCGTGG AGCTATTTTA GCAGGTACTG TTGATCTCAA
 1401 CGAAACAATT AGCGAAGTTG CCTTTAAGCA GAATACAGCA GCTCTAACTG
 1451 GAGGTGCTTT AAGTGCAAAAT GATAAGGTTA TAATTGCAAA TAACCTTTGGA
 1501 GAAATCTTTT TTGAGCAAAA CGAAGTGAGG AATCAGGAG GAGCCATTTA
 1551 TTGTGGATGT CGATCTAATC CTAAGTTAGA ACAAAGGAT TCTGGAGAGA
 1601 ACATCAATAT TATTGGAAC TCCGGAGCTA TCACTTTTTC AAAAAATAAG
 1651 GCTTCTGTTT TAGAAGTGAT GACACAAGCT GAAGATTATG CTGGTGAGG
 1701 CGCTTTATGG GGGCATAATG TTCTTCTAGA TTCCAATAGT GGAATATTC
 1751 AATTTATAGG AAATATAGGT GGAAGTACCT TCTGGATAGG AGAATATGTC
 1801 GGTGGTGGTG CGATCTCTC TACTGATAGA GTGACAATTT CTAATAACTC
 1851 TGGAGATGTT GTTTTAAAG GAAACAAAG CCAATGTCTT GCTCAAAAAT
 1901 ATGTAGCTCC TCAAGAAACA GCTCCCGTGG AATCAGATGC TTCATCTACA
 1951 AATAAAGACG AGAAGAGCCT TAATGCTTGT AGTCATGGAG ATCATTTATCC
 2001 TCTTAAACT GTAGAAGAGG AAGTGCCACC TTCATTGTTA GAAGAACATC
 2051 CTGTTGTTTC TTCGACAGAT ATTGTTGGTG GTGGGGCCAT TCTAGCTCAA
 2101 CATATCTTTA TTACAGATAA TACAGGAAAT CTGAGATTCT CTGGGAACCT
 2151 TGGTGGTGGT GAAGAGTCTT CTACTGTCCG TGATTTAGCT ATCGTAGGAG
 2201 GAGGTGCTTT GCTTTCTACT AATGAAGTTA ATGTTTGCAG TAACCAAAAT
 2251 GTTGTPTTTT CTGATAACGT GACTTCAAAT GGTGTGATT CAGGGGGAGC
 2301 TATTTTAGCT AAAAAAGTAG ATATCTCCGC GAACCACTCG GTTGAATTG

2351 TCTCTAATGG TTCAGGGAAA TTCGGTGGTG CCGTTTGC GC TTTAAACGAA
 2401 TCAGTAAACA TTACGGACAA TGGCTCGGCA GTATCATTTCT CTA AAAAATAG
 2451 AACACGTCTT GCGGTTGCTG GAGTTGCAGC TCCTCAAGGC TCTGTAACGA
 2501 TTTGTGGAAA TCAGGGAAAC ATAGCATTTA AAGAGAACTT TGT TTTTGGC
 5 2551 TCTGAAAAATC AAAGATCAGG TGGAGGAGCT ATCATTGCTA ACTCTTCTGT
 2601 AAATATTTCAG GATAACGCAG GAGATATCCT ATTTGTAAGT AACTCTACGG
 2651 GATCTTATGG AGGTGCTATT TTTGTAGGAT CTTTGGTTGC TTC TGAAGGC
 2701 AGCAACCCAC GAACGCTTAC AATTACAGGC AACAGTGGGG ATATCCTATT
 2751 TGCTAAAAATC AGCACGCAAA CAGCCGCTTC TTTATCAGAA AAAGATTCTT
 10 2801 TTGGTGGAGG GGCCATCTAT ACACAAAACC TCAAAATTGT AAAGAATGCA
 2851 GGGAAACGTTT CTTTCTATGG CAACAGAGCT CCTAGTGGTG CTGGTGTCCTA
 2901 AATTGCAGAC GGAGGAACATG TTTGTTTAGA GGCTTTTGGG GGAGATATCT
 2951 TATTTGAAGG GAATATCAAT TTTGATGGGA GTTTCAATGC GATTCACPTA
 3001 TCGGGGAATG ACTCAAAAAAT CGTAGAGCTT TCTGCTGTTT AAGATAAAAA
 15 3051 TATTATTTTC CAAGATGCAA TTACTTATGA AGAGAACACA ATTCTGCGCT
 3101 TGCCAGATAA AGATGTCAGT CCTTAAAGTG CCCCTTCATT AATTTTAAAC
 3151 TCCAAGCCAC AAGATGACAG CGCTCAACAT CATGAAGGGA CGATACGGTT
 3201 TTCTCGAGGG GTATCTAAAA TTCTCAGAT TGCTGCTATA CAAGAGGGAA
 3251 CCTTAGCTTT ATCACA AAAC GCAGAGCTTT GGT TGGCAGG ACTTAAACAG
 20 3301 GAAACAGGAA GTTCTATCGT ATTGCTGCG GGATCTATTC TCCGTATTTT
 3351 TGATTC CCAG GTTGATAGCA GTGCGCTCT TCCTACAGAA AATAAAGAGG
 3401 AGACTCTTGT TCTGCGCGGA GTTCAAATTA ACATGAGCTC TCCTACACCC
 3451 AATAAAGATA AAGCTGTAGA TACTCCAGTA CTTGCAGATA TCATAAGTAT
 3501 TACTGTAGAT TTGTCTTCAT TTGTTCCTGA GCAAGACGGA ACTCTTCCTC
 25 3551 TTCTCCTGA AATTATCATT CCTAAGGGAA CAAAATTACA TTCTAATGCC
 3601 ATAGATCTTA AGATTATAGA TCCTACCAAT GTGGGATATG AAAATCATGC
 3651 TCTTCTAAGT TCTCATAAAG ATATTCCATT AATTTCTCTT AAGACAGCGG
 3701 AAGGAATGAC AGGGACGCTT ACAGCAGATG CTTCTCTATC TAATATAAAA
 3751 ATAGATGTAT CTTTACCTTC GATCACACCA GCAACGTATG GTCACACAGG
 30 3801 AGTTTGGTCT GAAAGTAAAA TGGAAGATGG AAGACTTGTG GTCGGTTGGC
 3851 AACCTACGGG ATATAAGTTA AATCCTGAGA AGCAAGGGGC TCTAGTTTTG
 3901 AATAATCTCT GGAGTCATTA TACAGATCTT AGAGCTCTTA AGCAGGAGAT
 3951 CTTTGCTCAT CATACGATAG CTCAAAGAAT GGAGTTAGAT TTCTCGACAA
 4001 ATGCTCGGGG ATCAGGATTA GGTGTGTGTG AAGATTGTCA GAACATCGGA
 35 4051 GAGTTTGATG GGTTCAAACA TCATCTACA GGGTATGCC TAGGCTTGGG
 4101 TACACA AACT GTTGAAGACT TCTTAATTGG AGGATGTTTC TCACAGTTCT
 4151 TTGGTAAAC TGAAAGCCAA TCCTACAAAG CTAAGAACGA TGTGAAGAGT
 4201 TATATGGGAG CTGCTTATGC GGGGATTTTA GCAGGTCCCT GGTTAATAAA
 4251 AGGAGCTTTT GTTTACGGTA ATATAAACAA CGATTTGACT ACAGATTACG
 40 4301 GTACTTTAGG TATTTCAACA GGTTCATGGA TAGGAAAAGG GTTTATCGCA
 4351 GGCACAAGCA TTGATFACCG CTATATTGTA AATCCTCGAC GGT TATATC
 4401 GGCAATCGTA TCCACAGTGG TTCTTTTGT AGAAGCCGAG TATGTCCGTA
 4451 TAGATCTTCC AGAAATTAGC GAACAGGGTA AAGAGGTTAG AACGTTCCAA
 4501 AAAACTCGTT TTGAGAATGT CGCCATTCTT TTTGGATTTG CTTTGAACA
 45 4551 TCTTATTCG CGTGGCTCAC GTGCTGAAGT GAACAGTGT CAGCTTGCTT
 4601 ACGTCTTTGA TGTATATCGT AAGGGACCTG TCTCTTTGAT TACACTCAAG
 4651 GATGCTGCTT ATTC TTGGAA GAGTTATGGG GTAGATATTC CTTGTAAAGC
 4701 TTGGAAGGCT CGCTTGAGCA ATAATACGGA ATGGAATTCA TATTTAAGTA
 4751 CGTATTTAGC GTTTAATTAT GAATGGAGAG AAGATCTGAT AGCTTATGAC
 50 4801 TTCAATGGTG GTATCCGTAT TATTTTCTAG

The PSORT algorithm predicts an inner membrane location (0.106).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 42A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 42B) and for FACS analysis (Figure 42C). A his-tagged protein was also expressed.

55 The cp7287 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7287 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 43

The following *C.pneumoniae* protein (PID 4377105) was expressed <SEQ ID 85; cp7105>:

```

1  MSLYQXWVNS QLKSLCYST VAALIFMIPS QESFADSLID LNLGLDPSVE
51  CLSGDGAFSV GYFTKAGSTP VEYQPFKYDV SKRTFTILSV ETANQSGYAY
101 GISYDGTITV GTCSLGAGKY NGAKWSADGT L/PLTGTITGG TSHTEARAIS
151 KDTQVIEGFS YDASGQPKAV QWASGATTVT QLADISGGSR SSYAYALSDD
201 GTIIVGSMES TITRKTAVK WNNVPTYLG TLGGDASTGL YISGDGTIVT
251 GAANTATVTN GNQESHAYMY KDNQMKD*

```

The cp7105 nucleotide sequence <SEQ ID 86> is:

```

10 1  GTGAGTCTAT ATCAAAAATG GTGGAACAGT CAGTTAAAGA AGAGCCTCTG
51  CTATTCGACT GTTGCTGCTC TAATATTTAT GATTCCCTCT CAAGAATCCT
101 TTGCAGATAG TCTTATAGAT TTAAATTTAG GTTTAGATCC TTCGGTCGAA
151 TGTCTGTCTAG GAGATGGTGC ATTTTCTGTT GGGTATTTTA CTAAGGCGGG
201 ATCGACTCCC GTAGAATATC AGCCGTTTAA ATACGACGTA TCTAAGAAGA
15 251 CATTCACAAT CCTTTCGTA GAAACGGCAA ATCAGAGCGG CTATGCTTAC
301 GGAATCTCCT ACGATGGCAC GATCACTGTA GGAACGTGTA GCCTAGGTGC
351 AGGAAAATAT AACGGCGCAA AATGGAGTGC GGATGGCACT TTAACACCCT
401 TAACTGGAAT CACGGGGGGG ACGTCACATA CGGAAGCGCG TCGATTCTCT
451 AAGGATACTC AGGTGATCGA GGGTTTCTCA TATGATGCTT CAGGGCAACC
20 501 CAAGGCTGTG CAGTGGGCAA GCGGAGCGAC TACAGTAACA CAATTAGCAG
551 ATATTTTCAGG AGGCTCTAGA AGCTCTTATG CGTATGCTAT ATCTGATGAT
601 GGCACGATTA TTGTTGGGTC TATGGAGAGC ACGATAACAA GGAAACTACT
651 AGCTGTAAAA TGGGTAAATA ATGTTCTTAC GTATCTGGGA ACCTTAGGAG
701 GAGATGCTTC TACAGGTCTT TATATTTCTG GAGACGGCAC CGTGATTGTA
25 751 GGTGCGGCAA ATACAGCAAC TGTAACCAAT GGAATCAGG AATCCCACGC
801 CTATATGTAT AAAGATAACC AAATGAAAGA TTGA

```

The PSORT algorithm predicts an inner membrane location (0.100).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 43A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 43B) and for FACS analysis (Figure 43C). A his-tagged protein was also expressed.

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7105 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 44

The following *C.pneumoniae* protein (PID 4376802) was expressed <SEQ ID 87; cp6802>:

```

1  MSNQLQPCIS LGCVSYINSF PLSLQLIKRN DIRCVLAPPA DLLNLLIEGK
51  LDVALTSSLG AISHNLGYVP GFGIAANQRI LSVNLYAAPT FFNSPQPRIA
101 ATLESRSSIG LLKVLCRHLW RIPTPHILRF ITTKVLRQTP ENYDGLLLIG
40 151 DAALQHPVLP GFVTYDIASG WYDLTKLPFV FALLLHSTSW KBHPLPNLAM
201 EEALQQFESS PEEVLKEAHQ HTGLPPSLIQ EYYALCQYRL GEEHYESFEK
251 FREYYGTLYQ QARL*

```

A predicted signal peptide is highlighted.

The cp6802 nucleotide sequence <SEQ ID 88> is:

```

45 1  ATGTCTAACC AACTCCAGCC ATGTATAAGC TTAGGCTGCG TAAGTTATAT
51  TAATTCCTTT CCGCTGTCCC TACAATCAT AAAAAAGAAC GATATTCGCT
101 GTGTTCTTGC TCCCCGTGCA GACCTCCTCA ACTTGCTAAT CGAAGGAAAA
151 CTCGATGTTG CTTTGACCTC ATCCCTAGGA GCTATCTCTC ATAAC TTGGG
201 GTATGTCCCC GCCTTTGGAA TTGCAGCAAA CCAACGTATC CTCAGTGTA

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251 ACCTCTATGC AGCTCCCACT TTCTTTAACT CACCGCAACC TCGGATTGCC
 301 GCAACTTTAG AAAGTCGCTC CTCTATAGGA CTCTTAAAAG TGCTTTGTCTG
 351 TCATCTCTGG CGCATCCCAA CTCCTCATAT CCTAAGATTC ATAAC TACAA
 401 AAGTACTCAG ACAAACCCCT GAAAATTTATG ATGGCCTCCT CCTAATCGGA
 451 GATGCAGCGC TACAACATCC TGTACTTCCT GGATTGTAA CCTATGACCT
 501 TGCCTCGGGG TGGTATGATC TTACAAAGCT ACCTTTTGTA TTGCTCTTC
 551 TTCTACACAG CACCTCTTGG AAAGAACATC CCCTACCCAA CCTTGGCGATG
 601 GAAGAAGCCC TCCAACAGTT CGAATCTTCA CCCGAAGAAG TCCTTAAAGA
 651 AGCTCATCAA CATAACAGTC TGCCCCCTTC TCTTCTTCAA GAATACTATG
 701 CCCTATGCCA GTACCGTCTA GGAGAAGAAC ACTACGAAAG CTTTGAAAAA
 751 TTCCGGAAT ATTATGGAAC CCTCTACCAA CAAGCCGAC TGTA

The PSORT algorithm predicts an inner membrane location (0.060).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 44A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot

15. (Figure 44B) and for FACS analysis (Figure 44C). A his-tagged protein was also expressed.

These experiments show that cp6802 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 45

The following *C.pneumoniae* protein (PID 4376390) was expressed <SEQ ID 89; cp6390>:

20 1 MVFSYYCMGL FFFSGAISSC GLLVSLGVGL GLSVLGVLLL LLAGLLLFKI
 51 QSMLEREVPKA PDLLDLEDAS ERLRVKASRS LASLPKEISQ LESYIRSAAN
 101 DLNTIKTTPH KDQRLVETVS RKLERLAAQ NYMISELCEI SEILREEHH
 151 LILAQESLEW IGKSLFSTFL DMESFLNLSH LSEVRPYLAV NDPRLLEITE
 201 ESWEVVSHPF NVTSAFKKAQ ILFKNNEHSR MKKLLRSVQE LLETFLYKSL
 25 251 KRSYRELGCL SEKMRILHDN PLFPWVQDQ KYAHARNEFG RIARCLEEFE
 301 KTFFWLDEEC AISYMDCWDF LNESIQNKKS RVDRDYISTK KIALKDRART
 351 YAKVLLLENP TTEGKIDLQD AQRAPERQSQ EFTYLEHTET KVRLEALQQC
 401 FSDLREATNV RQVRFTNSEN ANDLKESEFK IDKERVYQK EQLYWETID
 451 RNEQELREBI GESLRLQNR KGYRAGYDAG RLKGLLRQWK KNLRDVEAHL
 30 501 EDATMDFEHE VSKSELCSVR ARLEVLEEEI MMSPKVADI KELLSEERC
 551 ILPIRENLER AYLQYNKCE ILSKAKFFFP EDEQLLVSEA NLREVGQOLK
 601 QVQGKCQERA QKFAIFEKHI QEQKSLIKEQ VRSFDLAGVG FLKSELLSIA
 651 CNLYIKAVVK ESIPVDVPCM QLYYSYEDN EAVVRNRLN MTERYQNFKR
 701 SLNSIQFNGD VLLRDPVYQP BGHETRLKER ELQETLSCK KLKVAQDRLS
 35 751 KLESRLSRR

A predicted signal peptide is highlighted.

The cp6390 nucleotide sequence <SEQ ID 90> is:

40 1 TTGGTATTCT CATACTATTG CATGGGATTA TTTTTTTTCT CTGGAGCTAT
 51 TTCTAGTTGT GGTCTTTTAG TGTCTCTAGG AGTTGGTTTA GGACTTAGTG
 101 TTTTAGGAGT ACTTTTACTT CTCCTTAGCAG GTCTTTTGCT TTTTAAGATC
 151 CAAAGTATGC TTCGAGAGGT GCCTAAGGCT CCTGATCTAT TAGATTTAGA
 201 AGATGCAAGT GAACGGCTTA GAGTAAAGGC TAGCCGTCTT TTAGCAAGCC
 251 TCCCGAAGGA AATCAGTCAG CTAGAGAGCT ACATTCGTTC TGCAGCTAAT
 301 GATCTAAATA CAATTAAGAC TTGGCCGCAT AAAGATCAAA GACTCGTCGA
 45 351 GACCGTGTCA CGAAAATTAG AGCGTCTGGC AGCTGCTCAA AACTATATGA
 401 TTTCTGAAC CTGCGAGATT AGTGAGATTC TTGAGGAAGA GGAGCATCAT
 451 CTAATTTTGG CTCAGGAATC TCTAGAATGG ATAGGTAAGA GTCTATTTTC
 501 TACCTTTCTG GACATGGAAT CTTTTTTAAA TTTGAGCCAT CTATCTGAAG
 551 TGCGTCCGTA CTTAGCTGTA AATGATCCTA GATTATTAGA AATTACCGAA
 50 601 GAATCTTGGG AAGTAGTGAG TCATTTTATA AATGTAACGT CTGCTTTTAA
 651 GAAAGCTCAG ATTCTTTTAA AGAACAACGA ACATTCCTCG ATGAAGAAGA
 701 AGTTAGAAAG TGTTCAGAG TTAGTGAAA CATTTATTTA TAAGAGTTTA
 751 AAGAGAAGTT ATCGAGAAAT AGGATGCTTA AGTGAAAAGA TGAGAATCAT
 801 TCACGACAAT CCTCTCTTCC CTTGGGTGCA AGATCAGCAG AAGTATGCTC
 55 851 ATGCTAAGAA TGAATTTGGA GAGATGCGC GGTGTTTAGA GGAGTTTGAA
 901 AAGACCTTCT TCTGGTTGGA TGAGGAGTGT GCTATTTCTT ACATGGACTG

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951 TTGGGATTTT CTAAATGAGT CTATTCAGAA TAAGAAGTCC AGAGTAGATC
 1001 GAGATTATAT ATCCACGAAG AAAATTGCAT TAAAGGATAG AGCCCGCACT
 1051 TATGCTAAGG TTCTTTTAGA AGAGAATCCG ACTACAGAGG GTAAAAATAGA
 1101 TTTGCAAGAC GCTCAAAGAG CCTTTGAGCG TCAAAGTCAG GAGTTTATA
 5 1151 CACTAGAGCA TACGGAAACA AAGGTGAGAC TAGAAGCACT TCAACAGTGC
 1201 TTCTCGGATC TTAGGGAGGC GACGAACGTA AGGCAAGTTA GGTTTACAAA
 1251 TTCTGAAAAT GCGAATGATT TAAAGGAGAG TTTGAGAGAAG ATAGATAAAG
 1301 AGCGTGTGCG ATATCAAAAA GAGCAAAGGC TCTATTGGGA AACAAATAGAT
 1351 CGCAATGAGC AAGAGCTTAG GGAAGAGATT GGGGAGTCGC TTCGTTTACA
 10 1401 AAATCGGAGA AAAGGGTATA GGGCTGGATA TGATGCTGGG CGTTTAAAAG
 1451 GTTTGTTGCG TCAGTGAAG AAAAATCTCC GCGATGTGGA AGCCACCTT
 1501 GAAGATGCAA CTATGGATT TGAAGCAAGA GCGAATGTG
 1551 CAGTGTTCGG GCGAGGCTCG AGGTCTAGA AGAAGAGCTG ATGGATATGT
 1601 CTCTTAAAGT TCGGATATA GAAGAGTTGT TGTCTTATGA AGAGCGTTGT
 15 1651 ATCTTCTCTA TTAGGAAAAA TTTAGAAAGG GCATACCTCC AATATAATAA
 1701 GTGTTCTGAA ATTTTATCCA AGGCAAAGTT CTCTTTCCG GAAGACGAGC
 1751 AATTGCTAGT TTCGGAAGCG AATCTAAGAG AGGTGGGTGC CCAGTTAAAA
 1801 CAAGTACAGG GAAAATGTCA AGAGAGGGCC CAAAAGTTCC CAATATTGA
 1851 AAAGCATATT CAGGAGCAGA AAAGCCTTAT TAAAGAGCAA GTCCGGAGTT
 20 1901 TTGATCTAGC GGGAGTGGG TTTTAAAGA GTGAGCTTCT TAGTATTGCT
 1951 TGTAACCTTT ATATAAAGGC GGTGTTAAG GAGTCTATAC CAGTTGATGT
 2001 GCCTTGTATG CAGTTATATT ATAGTTATTA CGAAGATAAT GAAGCTGTAG
 2051 TCGGAAACCG CCTTTTAAAT ATGACGGAGA GGTATCAAAA TTTTAAAGG
 2101 AGTTTGAATT CCATACAATT TAATGGTGAC GTTCTTTTAC GGGATCCGGT
 25 2151 CTATCAACCT GAAGGTCATG AGACCAGGCT AAAGGAACGG GAGCTACAAG
 2201 AAACAACCTT GTCTTGTAAG AAATTTAAAG TGGCTCAAGA TCGTCTTTCT
 2251 GAATTAGAGT CAAGGCTGTC TAGGAGATAG

The PSORT algorithm predicts a periplasmic location (0.932).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 45A.

30 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 45B) and for FACS analysis (Figure 45C). A his-tagged protein was also expressed.

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

35 These experiments show that cp6390 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 46

The following *C.pneumoniae* protein (PID 4376272) was expressed <SEQ ID 91; cp6272>:

1 MRRCFLFLAS FVIMGSSADA LTHQEAIVKK NSYLSHFQSV SGIVTIEDGV
 51 LNIHNNLRIO ANKVYVENTV GQSLKLVAGH NVMVNYRAKT LVCDYLEEYE
 40 101 DTDSCLLTNG RFAMYPWFLG GSMITLTPET IVIRKGYIST SEGPKKDLCL
 151 SGDYLEYSSD SLLSIGKTTL RVCRIPIFLP PPFSIMPMEI PKPPINFRGG
 201 TGGFLGSYLG MSYSPISRKH FSSTFFLDSE FKHGVGMGFN LHCSQKQVPE
 251 NVFNMKSYIA HRLAIDMAEA HDYRLHGF CPTHKHVNFV GEYHLSDSWE
 301 TVADIFPNMF MLKNTGPTRV DCTWNDNYFE GYLTSVKNV SFQANQELP
 45 351 YLTLRQYPIS IYNTGVYLEN IVECGYLNFA PSDHIVGENF SSLRLAARPK
 401 LHKTVPPLPIG TLSSTLGSSL IYSDVPEIS SRHSQLSAKL QLDYRFLHLK
 451 SYIQRRHIE PFVTFITETR PLAKNEDHYI FSIQDAFHSN NLLKAGIDTS
 501 VLSKTNPRFP RIHAKLWTH ILSNTESKPT FPKTACELSL PFGKNTVSL
 551 DAEWIWKHC WDHMNIRWEW IGNDNVAMTL ESLHRSKYSI IKCDRENFIL
 50 601 DVSRPIDQLL DSPLSDHRNL ILGKLFVRPH PCWNYRLSLR YGWHRQDTPN
 651 YLEYQMILGT KIFEHWQLYG VYERREADSR FFFFLKLDKP KKPPF*

A predicted signal peptide is highlighted.

The cp6272 nucleotide sequence <SEQ ID 92> is:

1 ATGAAACGTT GCTTCTTATT TCTAGCTTCC TTGTTCTTA TGGGTTCCCTC

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51 AGCTGATGCT TTGACTCATC AAGAGGCTGT GAAAAAGAAA AACTCCTATC
 101 TTAGTCACTT TAAGAGTGT TCTGGGATTG TGACCATCGA AGATGGGGTA
 151 TTGAATATCC ATAACAACCT GCGGATACAA GCCAATAAAG TGTATGTAGA
 201 AAATACTGTG GGTCAAAGCC TGAAGCTTGT CGCACATGGC AATGTTATGG
 5 TGAACATATAG GGCAAAAACC CTAGTTTGTG ATTACCTAGA GTATTACGAA
 301 GATACAGACT CTTGTCTTCT TACTAATGGA AGATTGCGGA TGTATCCTTG
 351 GTTTCTAGGG GGGTCTATGA TCACTCTAAC CCCAGAAACC ATAGTCATTC
 401 GGAAGGGATA TATCTCTACC TCCGAGGGTC CCAAAAAGA CCTGTGCCTC
 451 TCCGGAGATT ACCTGGAATA TTCTTCAGAT AGTCTTCTTT CTATAGGGAA
 10 GACAACATTA AGGGTGTGTC GCATTCCGAT ACTTTTCTTA CCTCCATTTT
 551 CTATCATGCC TATGGAGATC CCTAAGCCTC CGATAAACTT TCGAGGAGGA
 601 ACAGGAGGAT TTCTGGGATC CTATTGGGG ATGAGCTACT CGCCGATTTT
 651 TAGGAAGCAT TTCTCCTCGA CATTTTTCTT GGATAGCTTT TTCAAGCATG
 701 CGCTCGGCAT GGGATTCAAC CTCCATTGTT CTCAGAAGCA GGTTCCTGAG
 15 AATGTCTTCA ATATGAAAAG CTATTATGCC CACCGCTTG CTATCGATAT
 801 GGCAGAAGCT CATGATCGCT ATCGCCTACA CGGAGATTTT TGCTTCACGC
 851 ATAAGCATGT AAATTTTCTT GGAGAATACC ATCTCAGCGA TAGTTGGGAA
 901 ACTGTTGCTG ACATTTTCCC CAACAACCTC ATGTTGAAAA ATACAGGCC
 951 CACACGTGTC GATTGCACTT GGAATGACAA CTATTGTGAA GGGTATCTCA
 20 CCTCTTCTGT TAAGGTAAAC TCTTTCCAAA ATGCCAACCA AGAGCTCCCT
 1001 TATTTAACAT TAAGGCAGTA CCCGATTCTT ATTTATAATA CGGGAGTGTA
 1051 CCTTGAAAAC ATCGTAGAAT GTGGGTATTT AAACCTTGGT TTTAGCGATC
 1101 ATATCGTTGG CGAGAATTTC TCTTCACTAC GTCTTGCTGC GCGCCCTAAG
 1151 CTCCATAAAA CTGTGCCTCT ACCTATAGGA ACGCTCTCCT CCACCCTAGG
 25 GAGTTCCTCTG ATTTACTATA GCGATGTTCC TGAGATCTCC TCGCGCCATA
 1201 GTCAGCTTTC CGCGAAGCTA CAACTTGATT ATCGCTTCTT ATTACATAAG
 1301 TCCTACATTC AAAGACGCCA TATTATAGAG CCGTTCGTTA CCTTCATTAC
 1351 AGAGACTCGT CCTCTAGCTA AGAATGAAGA TCATTATATC TTTTCTATTC
 1401 AAGATGCCTT TCACTCCTTA AACCTTCTGA AAGCGGGTAT AGATACCTCG
 30 GTACTGAGTA AGACTAACCC TCGATTCCCG AGAATCCATG CGAAGCTGTG
 1501 GACTACCCAC ATCTTGAGCA ATACAGAAAG CAAACCCACG TTTCCCAAAA
 1551 CTGCACTGCGA GCTATCTCTA CCTTTTGGAA AGAAAAATA AGTCTCCTTA
 1601 GATGCTGAAT GGATTGGA AAGCACTGT TGGGATCACA TGAACATACG
 1651 TTGGGAGTGG ATCGGAAATG ACAATGTGGC TATGACTCTA GAATCCCTGC
 35 ATAGAAGCAA ATACAGCCTG ATTAAGTGTG ACAGGGAGAA CTTTATTTTA
 1701 ATAGTTCAGC GTCCCATTTGA CCAGCTTTTA GACTCCCTTC TCTCTGATCA
 1751 TAGGAATCTC ATTTTAGGGA AATTATTTGT ACGACCTCAT CCCTGTGTGA
 1801 ATTACCGCTT ATCCTTACGC TATGGCTGGC ATCGCCAGGA CACTCCGAAC
 1851 TACCTAGAAT ACCAGATGAT TCTAGGGACG AAGATCTTCG AACATTGGCA
 1901 GCTCTATGGG GTGTATGAAC GCCGAGAAGC AGATAGTCGA TTTTCTTCT
 40 TCTTAAAGCT CGACAAACCT AAAAAACCTC CCTTCTAA

The PSORT algorithm predicts an outer membrane location (0.48).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 46A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot and for
 45 FACS analysis (Figure 46B). A his-tagged protein was also expressed.

This protein also showed good cross-reactivity with human sera, including sera from patients with
 pneumonitis.

These experiments show that cp6272 is a surface-exposed and immunoaccessible protein, and that it
 is a useful immunogen. These properties are not evident from the sequence alone.

50 Example 47

The following *C.pneumoniae* protein (PID 4377111) was expressed <SEQ ID 93; cp7111>:

1 MFEAVIADIQ AREILDSRGY PTLHVKVTTT TGSVGEARVP SGASTGKKEA
 51 LEFRDFTDSPR YQGKGVLAQV KNVKEILFPL VKGCSVYEQS LIDSLMMDSD
 101 GSPNKETLGA NAILGVSLAT AHAAAATLRR PLRYRLGGCF ACSLPCPMN
 55 151 LINGMHADN GLEFQEFMIR PIGASSIKEA VNMGADVFT LKLLHERGL
 201 STGVGDEGGF APNLASNEBA LELLLLAIEK AGFTPGKDIS LALDCAASSF

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251 YNVKTGTYDG RHYEQIAIL SNLCDRYPID SIEDGLAED YDGWALLTEV
301 LGKKVQIVGD DLFVTNPCLI LEGISNGLAN SVLIKPNQIG TLTEFVYAIK
351 LAQMAGYTTI ISHRSGETTD TTIADLAVAF NAGQIKTGSL SRSERVAKYN
401 RLMEIEEELG SEAIPTDSNV FSYEDSEE*

```

5 A predicted signal peptide is highlighted.

The cp7111 nucleotide sequence <SEQ ID 94> is:

```

1 ATGTTTGAAG CTGTCATTGC CGATATCCAG GCTAGGGAAA TCTTGGATTG
51 TCGCGGGTAT CCCACTTTAC ATGTTAAAGT AACCACTAGC ACAGGTTCTG
101 TTGGAGAAGC TCGGGTTCCT TCAGGAGCAT CCACAGGGAA AAAAGAAGCC
151 TTAGAGTTTC GTGATACAGA TTCTCCTCGT TATCAAGGCA AAGGGGTTTT
201 GCAAGCTGTA AAAACGTAA AAGAAATCTT TTTTCCCCTC GTCAAGGGAT
251 GTAGTGTFTA TGAGCAATCC TTAATTGATT CTCTGATGAT GGATTCTGAC
301 GGCTCTCCGA ACAAGAAAC TCTAGGGGCC AATGCTATTT TAGGAGTCTC
351 TCTAGCTACA GCACATGCAG CAGCAGCAAC ACTACGCAGA CCTCTGTATC
15 401 GTTATTTAGG AGGGTGTFTT GCCTGCAGTC TTCCCTGTCC TATGATGAAT
451 CTGATCAATG GAGGCATGCA TGCCGATAAC GGCTTGGAGT TCCAAGAATT
501 TATGATCCGT CCTATGGAG CCTCTTCCAT CAAAGAAGCT GTCAACATGG
551 GTGCTGACGT TTTTCATACT TTGAAAAAAT TACTCCATGA AAGAGGCTTA
601 TCTACTGGAG TGGGTGACGA AGGAGGCTTC GCCCGAATC TTGCTTCTAA
20 651 TGAAGAAGCT CTAGAGCTCC TATTGCTGGC TATTGAAAAA GCAGGCTTTA
701 CTCCAGGAAA AGATATATCG CTAGCCTTAG ACTGCGCAGC ATCCTCATTC
751 TATAACGTAA AAACAGGCAC GTATGATGGG AGGCATATG AAGAGCAAAT
801 CGCAATCCTT TCTAATTTAT GTGATCGCTA TCCTATAGAC TCCATAGAAG
851 ATGGTCTTGC TGAAGAAGAC TATGACGGGT GGGCCTTGT TAACTGAAGTT
25 901 CTTGGAGAAA AAGTACAGAT TGTGGGTGAT GACCTATTG TTACAAATCC
951 GGAATTAATA TTAGAGGGTA TTAGCAATGG ATTAGCGAAC TCTGTGTTGA
1001 TTAACCAAAA TCAGATAGGG ACGCTTACTG AAACAGTGTA TGCTATCAAG
1051 CTTGCGCAAA TGGCTGGCTA TACTACAATT ATTTCTCATC GCTCAGGAGA
1101 AACTACGGAC ACTACGATTG CAGATCTTGC TGTTCCTTTC AACGCCGGTC
30 1151 AAATCAAAAC AGGCTCTTTA TCACGTTCTG AGCGTGTTCG AAAATACAAT
1201 AGACTCATGG AAATGAAGA AGAGCTTGA TCCGAAGCAA TTTTCACAGA
1251 TTCTAATGTA TTTTCTTAC GAGGATTCT GAGGAATAG

```

The PSORT algorithm predicts an inner membrane location (0.100).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 47A.

35 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 47B) and for FACS analysis (Figure 47C). A his-tagged protein was also expressed.

The cp7111 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

40 These experiments show that cp7111 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 48

The following *C.pneumoniae* protein (PID 4455886) was expressed <SEQ ID 95; cp0010>:

```

1 MKSQFSWLVL SSTLACFTSC STVFAATAEN IGPDSDFDGS TNTGYTFKN
51 TTTGIDYTLT GDITLQNLGD SAALTRGCFS DTTELSFAG KGYSLSFLNI
45 101 KSSAEGAALS VTDDKNLSLT GFSSLTFLAA PSSVITTPSG KGAVKCGGDL
151 TFDNNGTILF KDYCEENG AISTKNLSLK NSTGSISFEG NKSSATGKKG
201 GAICATGTVD ITNNTAPTIF SNNIAEAAGG AINSTGNCTI TGNTSLVFSE
251 NSVTATAGNG GALSGDADVT ISGNQSVTFS GNQAVANGGA IYAKKLTLAS
301 GGGGVSPFLT IIVQGTAGN GGAISILAAG ECLSLSAEAGD ITFNGNAIVA
50 351 TTPQTTRKNS IDIGSTAKIT NLRAISGHSI FFYDPITANT AADSTDITLNL
401 NKADAGNSTD YSGSIVFSGE KLSSEDAKVA DNLSTLTKQP VTLTAGNLVL
451 KRGVTLDTKG FTQTAGSSVI MDAGTTLKAS TEEVTLTGLS IPVDSLGEKG
501 KVVIAASAAS KNVALSGPIL LLDNQGNAYE NHDLGKTQDF SFVQLSALGT

```

551 ATTTDVPAPV TVATPTHYGY QGTWGMTWVD DTASTPKTKT ATLAWNTNGY
 601 LPNPERQGPL VPNSLWGSFS DIQAIQGVIE RSALTLCSDR GFWAAGVANF
 651 LDKDKKGEKR KYRHKSGGYA IGGAAQTCSE NLISFAPCQL PGSDKDFLVA
 701 KNHTDTYAGA FYIQHITECS GFICLLDLKL PGWSHKLPLV LEGQLAYSHV
 751 SNDLTKKYTA YPEVKGSWGN NAFNMMLGAS SHSYPEYLHC FDTYAPYIKL
 801 NLTYIRQDSF SEKGTEGRSF DDSNLFNLSL PIGVKFEKFS DCNDFS YDLT
 851 LSYVPLIRN DPKCTTALVI SGASWETIAN NLARQALQVR AGSHYAFSPM
 901 FEVLGQVFVE VRGSSRIYNV DLGGKFQF*

A predicted signal peptide is highlighted.

10 The cp0010 nucleotide sequence <SEQ ID 96> is:

1 ATGAAATCGC AATTTTCCTG GTTAGTGCTC TCTTCGACAT TGGCATGTTT
 51 TACTAGTTGT TCCACTGTTT TTGCTGCAAC TGCTGAAAAT ATAGGCCCCCT
 101 CTGATAGCTT TGACGGAAGT ACTAACACAG GCACCTATAC TCCTAAAAAT
 151 ACGACTACTG GAATAGACTA TACTCTGACA GGAGATATAA CTCTGCAAAA
 201 CCTTGGGGAT TCGGCAGCTT TAACGAAGGG TTGTTTTCCT GACACTACGG
 251 AATCTTTAAG CTTTGCCGGT AAGGGGTACT CACTTTCTTT TTTAAATATT
 301 AAGTCTAGTG CTGAAGGCGC AGCACTTTCT GTTACAACCTG ATAAAAATCT
 351 GTCGCTAACA GGATTTTCGA GTCTTACTTT CTTAGCGGCC CCATCATCGG
 401 TAATCACAAC CCCCTCAGGA AAAGGTGCAG TTAATGTGG AGGGGATCTT
 451 ACATTTGATA ACAATGGAAC TATTTTATTT AAACAAGATT ACTGTGAGGA
 501 AAATGGCGGA GCCATTTCTA CCAAGAATCT TTCTTTGAAA AACAGCACGG
 551 GATCGATTTC TTTTGAAGGG AATAAATCGA GCGCAACAGG GAAAAAAGGT
 601 GGGGCTATTT GTGCTACTGG TACTGTAGAT ATTACAAATA ATACGGCTCC
 651 TACCCCTCTT TCGAACAATA TTGCTGAAGC TGCAGGTGGA GCTATAAATA
 25 701 GCACAGGAAA CTGTACAATT ACAGGGAATA CGTCTCTTGT ATTTTCTGAA
 751 AATAGTGTGA CAGCGACCGC AGGAAATGGA GGAGCTCTTT CTGGAGATGC
 801 CGATGTTACC ATATCTGGGA ATCAGAGTGT AACTTTCTCA GGAAACCAAG
 851 CTGTAGCTAA TGGCGGAGCC ATTTATGCTA AGAAGCTTAC ACTGGCTTCC
 901 GGGGGGGGGG GGGTATCTCC TTTTCTAACA ATAAATAGTCC AAGGTACCAC
 30 951 TGCAGTAAT GGTGAGCCA TTTCTATACT GGCAGCTGGA GAGTGTAGTC
 1001 TTTACGAGA AGCAGGGGAC ATTACCTTCA ATGGGAATGC CATTGTTGCA
 1051 ACTACACCAC AAACACAAA AAGAAATTC ATTGACATAG GATCTACTGC
 1101 AAAGATCAGC AATTTACGTG CAATATCTGG GCATAGCATC TTTTCTACG
 1151 ATCCGATTAC TGCTAATACG GCTGCGGATT CTACAGATAC TTTAAATCTC
 35 1201 AATAAGGCTG ATGCAAGTAA TAGTACAGAT TATAGTGGGT CGATTGTTT
 1251 TTTCTGGTGA AAGCTCTCTG AAGATGAAGC AAAAGTTGCA GACAACCTCA
 1301 CTCTACGCT GAAGCAGCCT GTAACCTAA CTGCAGGAAA TTTAGTACTT
 1351 AAACGTGGTG TCACTCTCGA TACGAAAGGC TTTACTCAGA CCGCGGGTTC
 1401 CTCTGTTATT ATGGATGCGG GCACAACGTT AAAAGCAAGT ACAGAGGAGG
 40 1451 TCACTTTAAC AGGTCTTTCC ATTCTGTAG ACTCTTTAGG CGAGGTAAG
 1501 AAAGTTGTAA TTGCTGCTTC TGCAGCAAGT AAAAATGTAG CCTTAGTGG
 1551 TCCGATTCTT CTTTGTGATA ACCAAGGGAA TGCTTATGAA AATCAGGACT
 1601 TAGGAAAAAC TCAAGACTTT TCATTGTGTC AGCTCTCTGC TCTGGTACT
 1651 GCAACAACCTA CAGATGTTCC AGCGGTTCCCT ACAGTAGCAA CTCCTACGCA
 45 1701 CTATGGGTAT CAAGTACTT GGGGAATGAC TTGGGTGAT GATACCGCAA
 1751 GCACTCCAAA GACTAAGACA GCGACATTAG CTTGGACCAA TACAGGCTAC
 1801 CTTCCGAATC CTGAGCGTCA AGGACCTTTA GTTCTAATA GCCTTTGGGG
 1851 ATCTTTTCA GACATCCAAG CGATTCAAGG TGTCTAGAG AGAAGTGCTT
 1901 TGACTCTTTG TTTCAGATCGA GGCTTCTGGG CTGCGGGAGT CGCCAAATTC
 50 1951 TTAGATAAAG ATAAGAAAGG GGAACACGC AAATACCGTC ATAAATCTGG
 2001 TGGATATGCT ATCGGAGGTG CAGCGCAAAC TTGTTCTGAA AACTTAATTA
 2051 GCTTTGCCTT TTGCCAATC TTTGGTAGCG ATAAAGATTT CTAGTCGCT
 2101 AAAAATCATA CTGATACCTA TGCAGGAGCC TTCTATATCC AACACATTAC
 55 2151 AGAATGTAGT GGGTTCTATG GTTGTCTCTT AGATAAAGT CCTGGCTCTT
 2201 GGAGTCATAA ACCCTCGTTC TTAGAAGGGC AGCTCGCTTA TAGCCACGTC
 2251 AGTAATGATC TGAAGACAAA GTATATGCGG TATCTGAGG TGAAAGGTTT
 2301 TTGGGGGAAT AATGCTTTTA ACATGATGTT GGGAGCTTCT TCTCATTTCT
 2351 ATCTTGAATA CCTGCATTGT TTTGATACCT ATGCTCCATA CATCAAAGT
 2401 AATCTGACCT ATATACGTCA GGACAGCTTC TCGGAGAAAG GTACAGAAGG
 60 2451 AAGATCTTTT GATGACAGCA ACCTCTTCAA TTTATCTTTG CCTATAGGGG
 2501 TGAAGTTTGA GAAGTTCTCT GATTGTAATG ACTTTTCTTA TGATCTGACT
 2551 TTTATCTTATG TTCCTGATCT TATCCGCAAT GATCCCAAAT GCACTACAGC
 2601 ACTTGTAAAT AGCGGAGCCT CTTGGGAAAC TTATGCAAT AACTTAGCAC
 2651 GACAGGCCTT GCAAGTGCCT GCAGGCAGTC ACTACGCCTT CTCTCCTATG
 65 2701 TTTGAAGTGC TCGGCCAGTT TGCTTTTGAA GTTCGTGGAT CCTCACGGAT

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2751 TTATAATGTA GATCTTGGGG GTAAGTCCA ATTCTAG

The PSORT algorithm predicts an outer membrane location (0.922).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 48A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot

5 (Figure 48B) and for FACS analysis (Figure 48C). A his-tagged protein was also expressed.

The cp0010 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp0010 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

10 Example 49

The following *C.pneumoniae* protein (PID 4376296) was expressed <SEQ ID 97; cp6296>:

```

1  MEEVSEYLQQ VENQLESCSK RLTKMETFAL GVRLEAKEEI ESIILSDVVN
51  RFEVLCRDIE DMLSRVEEIE RMLRMABLPL LPIKEALTKA FVQHNSCKEK
101 LTKVEPYFKE SPAYLTSEER LQSLNQTLQR AYKESQKVSF LESEVRACRE
151 QLKDQVRQFE TQGVSLIKEE ILFVTSTFRT KFSYHSFRLH VPCMRLYEY
201 YDDIDLERTR ARWMAMSERY RDAFQAFQEM LKEGLVERAQ ALRETEYWLY
251 REERKSKKKH*
```

The cp6296 nucleotide sequence <SEQ ID 98> is:

```

1  ATGGAGGAGG TGTCTGAGTA TCTTCAGCAA GTAGAAAATC AGTTGGAATC
20  CTGTTCCAAG CGATTAAACCA AGATGGAAAC TTTTGCCTTA GGTGTGAGGT
51  TGGAAAGCTAA AGAAGAGATA GAGTCTATCA TACTTPTCTGA TGTAAGTAAC
101 CGTTTTGAGG TTTTATGTAG AGATATTGAA GATATGCTAT CTCGAGTCGA
151 GGAGATAGAG CGGATGTTAC GTATGGCGGA GCTTCCTCTA CTTCCCTATAA
201 AAGAAGCGCT TACCAAGGCT TTTGTACAAC ATAACAGCTG TAAAGAGAAG
251 TTAACCAAGG TAGAGCCTTA CTTTAAAGAG AGCCCTGCAT ATCTAACTAG
301 TGAAGAGCGA TTGCAGAGTT TGAATCAGAC TTTACAACGT GCGTACAAAG
351 AGTCCCAAAA GGTTCAGGTT TTAGAATCGG AAGTGAGAGC CTGTCGAGAG
401 CAGCTTAAAG ATCAAGTAAG ACAGTTTGAA ACTCAAGGAG TGAGCTTGAT
451 AAAAGAAGAG ATTCTCTTTG TGAAGTAGTAC CTTTAGAACT AAATTTAGCT
501 ATCAATTCATT TCGATTACAT GTTCCTTGCA TGAGGTTGTA TGAGGAGTAT
551 TATGATGACA TTGATCTAGA GAGAACTCGA GCTCGATGGA TGGCGATGTC
601 TGAGAGGTAT AGAGATGCTT TTCAGGCATT CCAGGAGATG TTGAAGGAAG
651 GCCTAGTTGA AGAAGCTCAG GCTCTTAGAG AAACCGAGTA CTGGTTATAT
701 CGAGAGGAGA GAAAGAGTAA AAAGAAACAT TGA
751
```

35 The PSORT algorithm predicts a cytoplasmic location (0.523).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 49A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 49B) and for FACS analysis (Figure 49C). A his-tagged protein was also expressed.

These experiments show that cp6296 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 50

The following *C.pneumoniae* protein (PID 4376664) was expressed <SEQ ID 99; cp6664>:

```

1  MVLFAQASG RNRVKADAIV LPFWHFKDAK NAASFEEFE PSYLPALENF
51  QGKTGEIELL YSSPKAKEKR IVLLGLGKNE ELTSDVVFQT YATLTRVLRK
101 AKCSTVNIIL PTISELRLSA EEFLVGLSSG ILSLNYDYPR YNKVDRNLET
```

45

151 PLSKVTVIGI VPKMADAIFR KEAIFEGVY LTRDLVNRNA DEITPKKLAE
 201 VALNLGKEFP SIDTKVLGKD AIAKEKMGLL LAVSKGSCVD PHFIVVRYQG
 251 RPKSKDHTVL IGKGVTFDSG GLDLKPGKSM LTMKEDMAGG ATVLGILSAL
 301 AVLELPIINV GIIIPATENAI DGASYKMGDV YVGMSGLSVE ICSTDABEGR
 351 ILADAITYAL KYCKPTRIID FATLTGAMVV SLGEEVAGFF SNNDVLAEDL
 401 LEASAETSEP LWRLPLVKKY DKTLLHSDIAD MKNLGSNRAG AITAALEFLQR
 451 FLEESSVAWA HLDIAGTAYH EKEEDRYPKY ASGFGVRSIL YYLENSLSK*

The cp6664 nucleotide sequence <SEQ ID 100> is:

1 GTGGTTTAT TTCATGCTCA AGCCTCTGGG CGTAATCGTG TTAAGGCAGA
 51 TGCTATAGTC CTGCCCTTTT GGCATTTTAA GGATGCAAAA AATGCAGCTT
 101 CTTTGAAGC CGAGTTTGAA CCCTCGTATC TCCCCGCTTT AGAAAACTTT
 151 CAAGGAAAAA CCGGGGAGAT TGAACCTCTT TATAGTAGTC CTAAAGCTAA
 201 GGAAAAACGC ATTGTCCTCT TAGGCTTAGG GAAAAATGAA GAGCTCACCT
 251 CTGATGTTGT TTTCCAAACC TATGCGACAC TAACTCGTGT CTTACGTAAA
 15 301 GCAAAGTGTT CCACAGTCAA TATCATCTTA CCTACAATTT CTGAATTGCG
 351 GCTTTCGTCC GAAGAATTCT TAGTGGGGTT GTCCTCAGGA ATTTTCTCAT
 401 TAAACTATGA CTACCCACGT TATAATAAGG TAGATCGTAA TCTTGAAACT
 451 CCTCTTTCTA AAGTCACGGT TATCGGTATC GTTCCCAAAA TGGCGGATGC
 501 TATCTTTAGG AAAGAAGCAG CCATTTTCGA AGGCGTATAT CTCACTCGAG
 20 551 ATCTTGTAAG CAGGAATGCT GATGAAATTA CCCCTAAGAA ATTGGCAGAG
 601 GTTGCTCTGA ATCTGGGAAA AGAGTTCCTT AGTATTGATA CTAAGGTCTT
 651 GGGAAAAGAT GCCATCGCCA AAGAGAAAAT GGGACTCCTA TTGGCTGTTT
 701 CCAAGGGTTC TTGTGTGGAT CCACACTTTA TCGTTGTCCG TTATCAAGGA
 751 CGTCTTAAGT CTAAAGATCA CACCGTCTTG ATAGGAAAAG GGGTCACTTT
 25 801 TGACTCTGGA GGTTTAGACC TCAAGCCTGG AAAATCCATG CTTACTATGA
 851 AAGAAGACAT GGCAGGTGGG GCTACAGTCC TCGGGATTCT CTCGGCGTTA
 901 GCAGTTTATG AGCTTCCTAT AAATGTCACG GGGATCATTC CTGCTACAGA
 951 GAATGCTATC GATGGCGCCT CCTATAAAAT GGGAGATGTC TATGTAGGAA
 1001 TGTGCGGGCT TTCTGTTGAG ATTTGTAGTA CCGATGCTGA GGGACGTCTT
 30 1051 ATCCTCGCTG ATGCGATTAC ATATGCTTTA AAATATTGTA AACCACACG
 1101 TATTATAGAT TTTCGCACTC TAACAGGAGC TATGGTAGTC TCTCTAGGAG
 1151 AAGAGGTTGC AGGTTCCTTT TCCAATAACG ATGTTTTCAG TGAAGATCTT
 1201 TTAGAGCGCT CAGCCGAAAC CTCCGAGCCG TTATGGAGAC TTCTCTAGT
 1251 TAAGAAGTAT GATAAAACAT TGCATTCTGA TATTGCTGAT ATGAAAAATC
 35 1301 TAGGCAGTAA CCGTGCAGGG GCTATTACAG CAGCATTATC CTTGCAGAGA
 1351 TTTTGGGAAG AATCTTCGGT AGCTTGGGCA CATCTTGATA TTGCAGGTAC
 1401 TGCATATCAT GAAAAAGAAG AAGACCGTTA TCCAAATATG GCTTCAGGTT
 1451 TTGGTGTCG TTCTATCTT TATTACTTAG AAAATAGTCT TTCTAAGTAG

The PSORT algorithm predicts an inner membrane location (0.268).

40 The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 50A), as a his-tagged protein, and as a GST/His fusion. The proteins were used to immunise mice, whose sera were used in Western blot Western blot (50B) and FACS (50C) analyses.

The cp6664 protein was also identified in the 2D-PAGE experiment (Cpn0385) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

45 These experiments show that cp6664 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 51

The following *C.pneumoniae* protein (PID 4376696) was expressed <SEQ ID 101; cp6696>:

50 1 MTLIFVIIIV WCNAFLIKLC VIMGLQSRLO HCIEVSQNSN PDSQVKQFIY
 51 ACQDKFTLRQS VLKIFRYHPL LKIHDIARAV YLLMALEEGB DLGLSFLNVQ
 101 QYPSGAVELF SCGGFPWKGL PYPAEHAEFG LLLLQIAEFY RESQAYVSKM
 151 SHFQQALFDH QGSVFPSSLWS QENSRLLEKE TTLSQSFLFQ LGMQIHPEYS
 201 LEDPALGFWM QTRSSSAFV AASGCQSSLG AYSSGDVGVI AYGPCSGDIS
 251 DCYFYGCCGI AKEFVCQKSH QTTEISFLTS TGKPHPRNTG PSYLRDSYVH
 55 301 LPIRCKITIS DKQYRVHAAL AEATSAMTFS IFCKGKNCQV VDGPRLRSCS

351 LDSYKPGND IMILGENDAI NIVSASPYME IFALQGKEKF WNADFLINIP
401 YKEEGVMLIF EKKVTSEKGR FFTKMN*

A predicted signal peptide is highlighted.

The cp6696 nucleotide sequence <SEQ ID 102> is:

```

5      1  TTGACTCTAA TTTTGTGTAT TATTATCGTT TGGTGCAATG CTTTCTGTAT
      51  CAAATGTGTC GTGATAATGG GGCTGCAATC CAGGTACAA CATGTATAG
     101  AAGTGTCCCA GAATTCGAAC TTTGATTCAC AAGTAAAACA GTTATCTAT
     151  GCGTGCCAAG ATAAGACATT AAGGCAGTCT GTACTCAAGA TTTCCGCTA
     201  CCATCCTTTA CTAATAATTC ATGATATTGC TCGGGCCGTC TATCTTTTGA
    10      251  TGGCCTTAGA AGAAGCGCAG GATTTAGGCT TAAGCTTTT AAATGTACAG
     301  CAGTACCCCT CAGGTGCTGT AGAACTGTTT TCTTGTGGGG GATTTCCTTG
     351  GAAAGGATTA CCTTATCCTG CAGAACATGC GGAATTTGGC CTACTCCTGT
     401  TACAGATCGC AGAGTTTAT GAAGAGAGTC AGGCATACGT CTCTAAAATG
     451  AGTCATTTTC AACAGGCACT CTTTGATCAC CAAGGGAGCG TCTTCCCTC
    15      501  TCTCTGGAGC CAGGAGAACT CTCGACTCCT AAAAGAAAAG ACAACTCTTA
     551  GCCAATCGTT TCTCTTCCAA TTAGGAATGC AAATTCACCC AGAATACAGT
     601  CTTGAGGATC CTGCACTAGG GTTCTGGATG CAAAGAACGC GTTCTTCATC
     651  CGCTTTTGTA GCCGCTTCAG GATGTCAAAG TAGCTTGGGA GCGTATTCCT
     701  CAGGGGATGT CGGTGTTATC GCTTATGGAC CTTGCTCTGG AGACATTAGT
    20      751  GATTGTATT ATTCTGGATG TTGTGGAATC GCTAAAGAGT TCGTGTGCCA
     801  AAAATCTCAC CAACTACAG AGATTCTTTT TCTCACCTCT ACAGGAAAGC
     851  CTCATCCCAG AAATACGGGA TTTTCTTACC TTCGAGATTC CTATGTACAT
     901  CTGCCGATCC GCTGTAAGAT CACTATTTCC GACAAGCAAT ATCGCGTGCA
     951  CGCTGCGTTG GCTGAGGCCA CCTCTGCCAT GACGTTTCT ATTTCTGTA
    25      1001  AGGGGAAGAA TTGTCAGGTT GTTGACGGCC CTCGCTTGGC CTCTGTTC
    1051  CTAGATTCTT ATAAAGGTCC CGGAAACGAC ATTATGATTC TTGGGGAAAA
    1101  TGACGCAATC AACATTGTTT CTGCAAGTCC CTATATGGAA ATTTTGTCTT
    1151  TGCAAGGCAA AGAAAAATTT TGGAATGCAG ACTTTTGAT TAATATTCCT
    1201  TACAAAGAAG AGGGCGTCAT GTTAATTTT GAAAAAAG TGACCTCTGA
    30      1251  GAAAGGAAGA TTCTTTACGA AGATGAATTA A

```

The PSORT algorithm predicts an inner membrane location (0.463).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 51A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 51B) and for FACS analysis (Figure 51C). A his-tagged protein was also expressed.

35 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6696 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 52

40 The following *C.pneumoniae* protein (PID 4376790) was expressed <SEQ ID 103; cp6790>:

```

      1  MSEHRKSSKI IGIDLGTINS CVSVMEGGQA KVITSSEGTR TTPSIVAFKG
     51  NEKLVGIPAK RQAVTNPEKT LGSTKRFIGR KYSEVASEIQ TVPYTVTSGS
    101  KGDVAFVDG KQYTPBEIGA QILMKMKETA BAYLGETVTE AVITVPAYFN
    151  DSQRASTKDA GRIAGLDVKK IPEPTAAAL AYGIDKVGDK KIAVFDLGGG
    201  TFDISILEIG DGVFVFLSTN GDTLLGGDDF DEVIIKWMIE EFKKQEGIDL
    251  SKDNMALQRL KDAAEKAKIE LSGVSSTEIN QPFITMDAQG PKHLALTLTR
    301  AQFERLAASL IERTKSPCIK ALSDAKLSAK DDDVLLVGG MSRMPAVQET
    351  AKELFGKEPN KGVNPDEVVA IGAAIQGGVL GGEVKDVLLE DVIPLSLGIE
    401  TLGGVMTTLV ERNTTIPTQK KQIFSTAADN QPAVTIVVLQ GERPMARDNK
    451  EIGRFDLTDI PPAPRGHPQI EVSFDIDANG IFHVSADIVA SGKEQKIRIE
    501  ASSGLQDEI QRMVRDAEIN KEEDKKRREA SDAKNEADSM IFRAEKAID
    551  YKEQIPETLV KEIEERIENV RNALKDDAPI EKIKEVTEDL SKHMQKIGES
    601  MQSQSASAAA SSAANAKGGP NINTEDLKKH SFSTKPPSNN GSSEDHIEEA

```

651 DVBIIDNDDK*

The cp6790 nucleotide sequence <SEQ ID 104> is:

```

      1  ATGAGTGAAC  ACAAAAAATC  AAGCAAATTT  ATAGGTATAG  ACTTAGGCAC
5      51  AACAAACTCC  TGGGTATCTG  TTATGGAAGG  AGGACAAGCT  AAAGTAATTA
      101  CATCATCCGA  AGGAACAAGA  ACCACGCCAT  CGATCGTTGC  CTTCAAAGGT
      151  AATGAGAAAT  TAGTGGGGAT  TCCAGCAAAA  CGTCAAGCAG  TGACAAATCC
      201  AGAAAAAACT  CTCGGCTCTA  CAAAACGCTT  TATTGGCCGT  AAGTACTCTG
      251  AAGTAGCTTC  GGAATCCAA  ACCGTTCCTT  ATACAGTCAC  CTCCGGATCT
      301  AAAGGTGATG  CCGTTTTCGA  AGTTGATGGC  AAACAATACA  CTCCAGAAGA
10     351  AATTGGCGCA  CAAATCTTAA  TGAAAATGAA  AGAGACAGCA  GAAGCTTATC
      401  TAGGCGAAAC  TGTACAGAA  GCAGTGATCA  CCGTCCCCGC  ATACTCAAT
      451  GATTCTCAAC  GAGCATCCAC  AAAAGATGCT  GGACGCATTG  CAGGTCTAGA
      501  TGTAACACGT  ATCATTCAG  AACCTACCGC  AGCAGCTCTT  GCCTACGGAA
      551  TCGATAAAGT  CCGTGATAAA  AAAATCGCTG  TCTTCGACCT  TGGTGGAGGA
15     601  ACTTTTGATA  TCTCCATCCT  AGAAATCGGT  GATGGCGTCT  TCGAAGTTCT
      651  ATCTACAAAT  GGAGATACTC  TCCTCGGTGG  AGACGACTTT  GATGAAGTCA
      701  TTATCAAATG  GATGATCGAA  GAATTCAAAA  AACAAGAAGG  CATTGATCTT
      751  AGCAAGATA  ATATGGCCTT  ACAAAGACTT  AAAGATGCTG  CTGAGAAAGC
      801  AAAAAATAGAA  CTTTCAGGAG  TCTCTTCCAC  AGAAATCAAT  CAGCCATTCA
20     851  TCACAATGGA  TGCACAAGGA  CCTAAACACC  TTGCATTGAC  ACTCACACGT
      901  GCGCAATTCG  AGAACTCGC  AGCCTCTCTA  ATCGAAAGAA  CAAAATCTCC
      951  ATGCATCAAA  GCACTCAGTG  ACGCAAAACT  TTCCGCTAAG  GATATCGATG
100    1001  ATGTCTCTTT  AGTTGGAGGT  ATGTCAAGAA  TGCCCGCAGT  GCAAGAAACT
      1051  GTAAAGAAC  TCTTCGGCAA  AGAGCCTAAT  AAAGGAGTCA  ACCCGACGA
25     1101  AGTTGTTGCT  ATTGGAGCCG  CAATTCAAGG  TGGTGTCTTT  GGCGGAGAAG
      1151  TTAAGGATGT  TCTACTTCTA  GACGTTATCC  CCCTATCTCT  GGGTATCGAA
      1201  ACTCTAGGAG  GCGTCATGAC  GACTCTGGTA  GAGAGAAATA  CTACAATCCC
      1251  TACACAGAAA  AAACAAATCT  TCTCCACAGC  TGCTGATAAC  CAGCCTGCGG
      1301  TTACCATCGT  AGTTCTCCAA  GGAGAGCGTC  CCATGGCCAA  AGATAACAAG
30     1351  GAAATCGGAA  GATTGATCT  TACAGATATC  CCTCCGGCTC  CTCGAGGCCA
      1401  TCCTCAAATC  GAAGTCTCCT  TCGATATCGA  TGCAACCGGA  ATTTTCCATG
      1451  TCTCAGCTAA  AGATGTTGCC  AGCGGTAAAG  AACAGAAAAT  TCGTATCGAA
      1501  GCAAGCTCAG  GACTTCAAGA  AGATGAAATC  CAAAGAATGG  TTCGAGATGC
      1551  CGAAATTAAT  AAGGAAGAAG  ATAAAAACG  TCGTGAAGCT  TCAGATGCTA
35     1601  AAAATGAAGC  CGATAGCATG  ATCTTCAGAG  CCGAAAAAGC  TATTAAAGAT
      1651  TATAAGGAGC  AAATTCCTGA  AACTTTAGTT  AAAGAAATCG  AAGAGCGAAT
      1701  CGAAACGCTG  CGCAACGCAC  TCAAAGATGA  CGCTCCTATT  GAAAAAATTA
      1751  AAGAGGTTAC  TGAAGACCTA  AGCAAGCATA  TGCAAAAAAT  TGGAGAGTCT
      1801  ATGCAATCGC  AGTCTGCATC  AGCAGCAGCA  TCATCGGCAG  CCAATGCTAA
40     1851  AGGTGGACCT  AACATCAATA  CAGAAGATT  T  GAAAAACAT  AGTTTCAGTA
      1901  CGAAGCCTCC  TTCAAATAAC  GGTTCCTFCAG  AAGACCATAT  CGAAGAAGCT
      1951  GATGTAGAAA  TTATTGATAA  CGACGATAAG  TAA

```

The PSORT algorithm predicts an inner membrane location (0.151).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 52A) and a his-tagged product. The proteins were used to immunise mice, whose sera were used in Western blot (Figure 52B) and FACS (Figure 52C) analyses.

The cp6790 protein was also identified in the 2D-PAGE experiment (Cpn0503).

These experiments show that cp6790 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

50 Example 53

The following *C.pneumoniae* protein (PID 4376878) was expressed <SEQ ID 105; cp6878>:

```

      1  MNVPDSKNLH  PPAYELLEIK  ARITQSYKEA  SAILTAIPDG  ILLLSETGHF
51  LICNSQAREI  LGIDENLEIL  NRSFTDVLDP  TCLGFSIQEA  LESLKVPKTL
101  RLSLCKESKE  KEVELFIRKN  EISGYLFIQI  RDRSDYKQLE  NAIRYKNIA
55  151  ELGKMTATLA  HEIRNPLSGI  VGFASILKKE  ISSPRHQRL  SSIISGTRSL
      201  NNLVSSMLEY  TKSQPLNLKI  INLQDFFSSL  IPLLSVSFPN  CKFVREGAQP

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251 LFRSIDPDRM NSVVWNLVKN AVETGNSPIT LTLHTSGDIS VTNPGTIPSE
 301 IMDKLFPPFF TTKREGNGLG LABAQKIIRL HGGDIQLKTS DSAVSFFIII
 351 PELLAALPKE RAAS*

The cp6878 nucleotide sequence <SEQ ID 106> is:

5 1 ATGAACGTCC CTGATTCCAA GAACCTCCAT CCTCCTGCAT ACGAACTCCT
 51 AGAGATCAAG GCTCGCATCA CACAATCTTA TAAAGAAGCG AGTGCTATAC
 101 TGACAGCGAT TCCTGATGGT ATCCTATTAC TTTCTGAAAC AGGACACTTT
 151 CTTATCTGCA ATTACACAAGC ACGTGAAATT CTAGGAATTG ATGAAAATCT
 201 AGAAATCTTT AATAGATCCT TTACCGATGT TCTCCCCGAT ACGTGTCTTG
 10 251 GATTTCCTAT TCAAGAGGCT CTTGAATCTC TAAAGATCCC TAAAACTCTT
 301 AGACTCTCTC TCTGTAAAGA ATCTAAAGAA AAAGAAGTGG AACTCTTCAT
 351 CCGTAAAAAC GAGATCAGTG GATACCTGTT TATCCAAATC CGCGATCGGT
 401 CCGACTATAA ACAACTAGAA AACGCTATAG AAAGATATAA AAATATCGCA
 451 GAACCTGGGA AAATGACGGC TACCCTAGCT CACGAAATCC GCAATCCGCT
 15 501 AAGTGGAAAT GTTGGATTTG CCTCTATCCT AAAGAAAGAG ATTTCTCTCT
 551 CTCGCCACCA ACGAATGCTC TCCTCAATCA TCTCCGGCAC AAGGTCTCTA
 601 AATAACCTTG TCTCTCTAT GTTAGAATAT ACAAAATCAC AACCGTGA
 651 CCTAAAGATT ATAAATTTAC AAGACTTCTT CTCTTCTCTT ATCCCTCTGC
 701 TCTCCGTCTC TTTCCGAAT TGCAAGTTTG TAAGAGAGGG CGCACAACT
 20 751 CTATTCAGAT CTATAGATCC TGATCGGATG AACAGTGTG TTTGGAACCT
 801 AGTGAAAAAT GCTGTAGAAA CAGGGAAGTC TCCGATCACT CTGACCCTGC
 851 ATACATCGGG AGACATCTCG GTAACGAACC CCGGAACGAT TCCTTCCGAG
 901 ATCATGGACA AGCTCTTCAC TCCATTCTTC ACAACAAAGA GAGAGGGAAA
 951 TGGTTTGGGA CTGCTGAAG CTCAAAAAAT TATAAGACTC CATGGAGGAG
 25 1001 ATATCCAATT AAAACAAGC GACTCCGCCG TTAGCTTCTT CATAATCATC
 1051 CCCGAACTTC TAGCGGCCCT ACCCAAAGAA AGAGCCGCTA G

The PSORT algorithm predicts an inner membrane location (0.204).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 53A) and as a GST-fusion product. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 53B) and for FACS analysis.

These experiments show that cp6878 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 54

The following *C.pneumoniae* protein (PID 4377224) was expressed <SEQ ID 107; cp7224>:

35 1 MMKIKRKVAL AVGGSGGHIV PALSVKEAFS REGIDVILLG KGLKNHPSLQ
 51 QGISYREIPS GLPTVLNPIK IMSRTLSLCS GYLKARKELK IFDPLDVIGF
 101 GSYHSLPVLV AGLSHKIPLF LHEQNLVPGK VNQLFSRYAR GIGVNFSPVT
 151 KHFRCPAEV FLPKRSFSLG SPMKRCRTH TPTICVVGGS QGAQILNFCV
 201 PQALVKLVNK YPNMYVHHIV GPKSDVMKVQ HVYNRGEVLC CVKPFEEQLL
 40 251 DVLLAADLVI SRAGATILEE ILWAKVPGIL IPYPGAYGHQ EVNAKFFVDV
 301 LEGGTMLEK ELTEKLLVEK VTFALDSHNR EKQRNSLAAY SQQRSTKTFH
 351 AFICECL*

The cp7224 nucleotide sequence <SEQ ID 108> is:

45 1 ATGATGAAGA AAATTCGAAA AGTAGCCTTG GCTGTAGGAG GTTCAGGAGG
 51 CCACATGTGC CCAGCTCTCT CGGTAAAGGA AGCTTTTCTT CGTGAAGGAA
 101 TAGACGTATT ACTACTAGGG AAAGGTCTCA AGAACCATCC TTCTTTGCAA
 151 CAGGGAATCA GCTATCGGGA AATCCCTTCA GGAATCCTA CAGTCCTTAA
 201 TCCCATAAAG ATCATGAGCA GGACCTTTC TCTATGTTCA GGATACCTGA
 251 AAGCAAGAAA GGAACCTAAA ATTTTGGACC CTGACCTGGT CATAGGATTT
 50 301 GGGAGCTACC ACTCTCTTCC CGTGTGCTC GCAGGACTGT CCCATAAAAT
 351 TCCCTTATTT CTACACGAAC AAAATCTAGT TCCTGGAAAA GTAATCAAT
 401 TGTTTTCCCG CTATGCTCGA GGTATTGGAG TGAATTTCTC CCCCCTTACT
 451 AAGCACTTCC GCTGCCCCGC AGAAGAGGTC TTCCTTCTTA AACGAAGCTT
 501 CTCCTTAGGA AGCCCTATGA TGAAGCGATG TACAAATCAT ACCCTACAA
 55 551 TCTGTGTTGT TGGAGGTTCT CAGGGAGCAC AGATATTAAA TACTTGTGTT
 601 CCCCAAGCTC TTGTCAAGCT AGTCAATAAG TACCCAAATA TGTACGTCCA

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651 TCATATTGTA GGACCTAAAA GTGATGTTAT GAAGGTGCAA CATGTTTACA
701 ATCGTGGAGA GGTCTCTGCG TGTGTGAAGC CGTTTGAAGA GCAACTCCTA
751 GATGTCTTGC TTGCCGCGAGA TTTGGTCATC AGTAGGGCAG GAGCCACAAT
801 TTTAGAAGAA ATTCTTTGGG CAAAAGTTCC CGGAATTTTA ATTCCCTATC
851 CAGGAGCTTA TGGACATCAG GAAGTTAATG CTAAATTCCT TGTAGACGTC
901 TTAGAAGGGG GAACTATGAT CCTAGAAAAA GAATTAACAG AGAAGCTATT
951 AGTAGAAAAA GTAACGTTTG CTTTAGACTC CCATAACAGA GAAAAACAAC
1001 GCAATTCCCT AGCGGCGTAT AGTCAGCAA GGTCAACAAA AACATTCCAT
1051 GCATTCATTT GTGAATGCTT ATAG

```

10 The PSORT algorithm predicts an inner membrane location (0.164).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 54A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 54B) and for FACS analysis (Figure 54C). A his-tagged protein was also expressed.

15 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7224 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 55

The following *C.pneumoniae* protein (PID 4377140) was expressed <SEQ ID 109; cp7140>:

```

20 1 MVRRSISFCL FFLMTLLCCT SCNSRSLIVH GLPGREANEI VVLLVSKGVA
51 AOKLPOAAAA TAGAATEQMW DIAVPSAQIT EALAILNQAG LPRMKGTSLL
101 DLFAKQGLVP SELQEKIRYQ EGLSEQMAST IRKMDGVVDA SVQISFTTEN
151 EDNLPLTASV YIKHRGVLDN PNSIMVSKIK RLIASAVPGL VPENVSUVSD
201 RAAYSDITIN GPWGLTEIID YVSVWGIILA KSSLTKFRLI FYVLILILFV
25 251 ISCGLLWVIW KTHTLIMTMG GTRGFFNPTP YTKNALEAKK AEGAAADKEK
301 KEDADSQGES KNAETSDKDS SDKDAPEGSN EIEGA*

```

A predicted signal peptide is highlighted.

The cp7140 nucleotide sequence <SEQ ID 110> is:

```

30 1 ATGGTTCGTC GATCTATTTT TTTTGTCTTG TCTTTCTTAA TGACATGCT
51 GTGCTGTACA AGCTGTAAAC GCAGGTCTCT AATTGTGCAC GGTCTTCCTG
101 GCAGAGAAGC GAATGAGATT GTGGTGCTTT TGGTAAGCAA AGGGGTGGCT
151 GCACAAAAAT TGCCTCAAGC TGCAGCGGCT ACAGCCGGAG CAGCTACTGA
201 GCAAATGTGG GATATCGCGG TTCCGTCAGC ACAAATCACA GAGGCCCTTG
25 251 CCATTCTAAA TCAAGCGGGT CTTCCACGTA TGAAAGGGAC AAGCCTGTTA
35 301 GATCTTTTTC CAAAACAAGG TCTTGTTCCT TCCGAGCTTC AGGAAAAAAT
351 CCGTTATCAA GAAGGCTTAT CAGAACAGAT GGCTCTACG ATTAGAAAAA
401 TGGATGGCGT TGTCGATGCC TCAGTACAGA TTTCTTTCAC TACAGAAAAA
451 GAAGATAATC TTCTTTTAAC AGCCTCTGTG TATATTAAGC ATCGAGGGGT
501 TTTGGACAAT CCGAACAGCA TTATGGTTTC CAAAATTAAG CGCCTTATTG
40 551 CAAGTGCTGT TCCAGGACTT GTGCCAGAGA ACGTCTCTGT AGTGAGCGAT
601 CGCGCAGCTT ATAGTGATAT TACAATTAAT GGTCTTGGG GATTACAGA
651 AGAAATCGAT TATGTTTCTG TTTGGGGTAT TATTCTTTCG AAGTCTTCGC
701 TCACCAAATP CCGTCTCATT TTTTATGTCT TGATTCTCAT TTTATTGTG
751 ATTTCTTGTG GTCTCCTTTG GGTCAATTGG AAAATCATA CTCTCATTAT
45 801 GACTATGGGA GGTACAAAAG GGTCTTTCAC CCCTACACCA TATACAAAAG
851 ATGCCTTGGA AGCCAAGAAA GCCGAGGGAG CAGCTGTCTG CAAAGAGAAA
901 AAAGAAGATG CAGATTACAC GGGGGAAGC AAAATGCGG AAACCAGTGA
951 TAAAGACTCT AGTGATAAAG ATGCTCCAGA AGGAAGCAAT GAAATTGAGG
1001 GTGCTTAG

```

50 The PSORT algorithm predicts an inner membrane location (0.650).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 55A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 55B) and for FACS analysis (Figure 55C). A his-tagged protein was also expressed.

These experiments show that cp7140 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 56

The following *C.pneumoniae* protein (PID 4377306) was expressed <SEQ ID 111; cp7306>:

```

1  MTTKQLRSWL AVLVGSSLLA LPLSGQAVGK KESRVSELPQ DVLLKEISGG
51  FSKVATKATP AVVYIESFPK SQAVTHPSPG RRGPYENPFD YFNDEFFNRF
101 FGLPSQREKP QSKEAVRG TG FLVSPDGYIV TNNHVVEDTG KIHVTLHDGQ
151 KYPATVIGLD PKTDLAVIKI KSONLPYLSF GNSDHLKVG D WAIAIGNPFG
201 LQATVTVGVI SAKGRNQLHI ADFEDFIQTD AAINPGNSGG PLLNIDGQVI
251 GVNTAIVSGS GGYIGIGFAI PSLMANRIID QLIRDGQVTR GFLGVTLQPI
301 DAELAACYKL EKVGALVTD VVKGSPADKA GLKQEDVIIA YNGKEVDSLS
351 MFRNAVSLMN PDTRIVLKVV REGKVIEIPV TVSQAPKEDG MSALQRVGIR
401 VQNLTPETAK KLGIAPETKG ILIISVEPGS VAASSGLAPG QLILAVNRQK
451 VSSIEDLNRT LKDSNNENIL LMVSQGDVIR FIALKPEE*
```

A predicted signal peptide is highlighted.

The cp7306 nucleotide sequence <SEQ ID 112> is:

```

1  ATGATAACTA AGCAATFGCG TTCGTGGCTA GCTGTACTTG TTGGTTCAAG
51  TCTGCTAGCT CTTCCTTTAT CAGGGCAAGC TGTCGGGAAA AAAGAATCTC
101 GAGTTTCCGA GCTGCCTCAA GACGTTCTTC TTAAAGAGAT CTCGGGAGGG
151 TTTTCTAAGG TCGCTACCAA GGCGACTCCC GCTGTGTGT ACATAGAAAG
201 TTTCCCAAAG AGCCAGGCTG TAACACATCC TTCTCCTGGA CGCCGTGGGC
251 CTTATGAAAT TCCTTTTGAT TATTTAATG ATGAGTTTTC CAATCGTTT
301 TTTGGTCTAC CTTACACAGG GGAAAAACCT CAAAGTAAAG AGGCGGTTCG
351 AGGAACAGGT TTCCTAGTAT CTCCAGATGG CTATATTGTG ACTAATAACC
401 ATGTTGTCGA AGATACAGGT AAGATTCACG TAACTCTTCA TGATGGGCAA
451 AAGTACCCAG CAACTGTAAT CGGACTCGAT CCTAAAACAG ACCTTGCGAT
501 CATTAAAATT AAATCCCAAA ACCTCCCGTA TCTTCTTTT GGAAACTCCG
551 ACCACTTAAA AGTCGGAGAT TGGGCAATTG CAATTGGAAA TCCCTTCGGT
601 CTTCAAGCTA CGGTCAACCGT AGGTGTCATC AGTGCTAAAG GAAGAAATCA
651 ACTCCACATT GCAGATTTTG AAGATTTTAT TCAGACAGAT GCTGCGATTA
701 ATCCAGGCAA CTCTGGAGGC CCTCTTCTAA ATATTGATGG ACAGGTCATC
751 GGTGTTAATA CTGCCATTGT CAGTGGTAGT GGTGGCTATA TTGGAATCGG
801 GTTTGCGATT CCTAGCCTTA TGGCAAATAG AATCATAGAT CAGCTGATTC
851 GTGATGGTCA AGTTACCCGA GGATTCCTAG GAGTGACTTT ACAACCTATA
901 GATGCGGAAC TCGCTGCTTG CTACAACTC GAAAAGGTTT ATGGCGCTTT
951 AGTCACAGAT GTTGTAAAG GATCTCCAGC AGATAAAGCA GGGCTAAAC
1001 AAGAAGATGT GATCATTGCT TATAATGGGA AAGAAGTCGA TTCACTGAGT
1051 ATGTTCCGTA ATGCTGTTTC TTAAATGAAT CCAGATACAC GTATTGTTCT
1101 AAAGGTAGTT CGTGAAGGAA AGGTTATCGA AATACCCGTG ACAGTTTCTC
1151 AAGCTCCAAA AGAAGATGGA ATGTCGGCTT TACAGCGTGT GGAATCCGT
1201 GTGCAAAACC TAACTCCTGA AACTGCTAAG AAGCTGGGAA TTGCTCCAGA
1251 GACTAAAGGC ATTTTGATTA TAAGTGTTGA ACCAGGCTCT GTAGCAGCTT
1301 CTTCAGGAAT TGCTCTGGT CAGCTGATCC TTGCTGTGAA TAGACAAAAA
1351 GTATCTTCGA TTGAAGATCT GAATAGAACG TTAAGATT CTAACAATGA
1401 GAATATCTT CTTATGTTT CTAAGGAGA TGTATTTCGC TTCATTGCC
1451 TGAAACCTGA AGAATAA
```

The PSORT algorithm predicts a periplasmic location (0.923).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 56A) and as a GST-fusion product (Figure 56B). The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 56C) and for FACS (Figure 56D) analyses.

The cp7306 protein was also identified in the 2D-PAGE experiment (Cpn0979) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7306 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

5 Example 57

The following *C.pneumoniae* protein (PID 4377132) was expressed <SEQ ID 113; cp7132>:

```

1  MCNSTIAMKKQ KRGFVLMELL MSFTLIAALLL GTLGFWYRKI YTVQKQKERI
51  YNFYIEESRA YKQLRTLFSM SLSSSYEEPG SLFSLIFDRG VYRDPKLAGA
101 VRASLHHDTK DQRLELRICN IKDQSYFETQ RLLSHVTHVV LSFQRNPDPB
151 KLPETIALTI TREPKAYPPR TLTYQFAVGK*

```

A predicted signal peptide is highlighted.

The cp7132 nucleotide sequence <SEQ ID 114> is:

```

1  ATGTGTAACCT CTATAGCTAT GAAAAAGCAA AAGCGTGGCT TTGTGCTTAT
51  GGAAATTACTC ATGTCGTICA CTCTAATTGC TTGTATTATTA GGGACTTTAG
101 GATTTTGGTA TCGGAAAATT TATACTGTAC AAAAGCAAAA AGAACGTATT
151 TATAACTTTT ATATCGAAGA AAGCCGAGCC TACAAGCAGC TCAGAACCCCT
201 GTTTAGCATG TCCTTGCTCT CATCTTACGA GGAGCCTGGA TCATTATTTT
251 CTTTAATCTT TGATCGGGGT GTTATCGAG ATCCTAAGCT GGCAGGTGCG
301 GTACGAGCTT CTCTCCATCA TGACACCAAG GATCAGAGAT TGGAACTTCG
351 TATTTGTAAT ATTAAGGATC AGTCTTACTT TGAAACACAG CGACTGCTCT
401 CCCACGTGAC CCATGTTGTA CTTTCCTTCC AGAGAAATCC TGATCCTGAA
451 AAACCTCCTG AAACAATTGC TTAACTATA ACACGGGAAC CTAAAGCATA
501 TCCTCCAAGG ACGTTAACAT ACCAATTGCG GGTGGGAAA TAA

```

The PSORT algorithm predicts a periplasmic location (0.915).

25 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 57A) or as a GST-fusion. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 57B) and FACS (Figure 57C) analyses.

These experiments show that cp7132 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

30 Example 58

The following *C.pneumoniae* protein (PID 4376733) was expressed <SEQ ID 115; cp6733>:

```

1  MKTSIPWVLV SSVLAFSCHL QSLANEELLS PDSFNGNID SGTFTPKTSA
51  TTYSLTGDVVF FYEPGKGTP L SDSCFKQTTD NLTF LGNGHS LTFGFI DAGT
101 HAGAAASTTA NKNLTFSGFS LLSFDSSPST TVTTGQGTLS SAGGVNLENI
35 151 RKLVVAGNFS TADGGAIKGA SFLLTGTS GD ALFSNNSSST KGGAIAT TAG
201 ARIANNFGYV RFLSNIASTS GGAIDDEGTS ILSNNKFLYF EGNAAKT TGG
251 AICNTRKASGS PELIISNNKT LIFASNVAET SGGAIHAKKL ALSSGGFTEF
301 LRNVSSATP KGAISIDAS GELSLSAETG NITFVRNLT TTGSTDT PKR
351 NAINIGSNKG FTELRAAKNH TIFYDPTTS EGTSSDVLKI NNGSAGALNP
40 401 YQGTILFSGE TLTADELKVA DNLKSSFTQP VSLSGGKLLL QRGVTLBSTS
451 FSQEAGSLLG MDSGTTLSTT AGSITITNLG INVDSLGLKQ PVSLTAKGAS
501 NKVIVSGKLN LIDIEGNIYE SHMFSDQLF SLLKITVDAD VDTNVDISSL
551 IPVPAEDPNS EYGFQGGWNV NWTDTATNT KBATATWTKT GFVPSPERKS
601 ALVCNTLWGV FTDIRSLQQL VEIGATGMEH KQGFVSSMT NFLHKTGDEN
45 651 RKGFRHTSGG YVIGGSAHTP KDDLFTFAPC HLFARDKDCF IAHNNSRTYG
701 GTLFFKHSHT LQPQNYLRLG RAKFSESAIE KFPREIPLAL DVQVSF SHSD
751 NRMETHYTS L PESEGSWSNE CIAGGIGLDL PFVLSNPHEL FKTFI PQMKV
801 EMVYVSQNSF FESSSDGRGF SIGRLNL LSI PVGAKFVQGD IGSYTYDLS

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851 GFFVSDVYRN NPQSTATLVM SPDSWKIRGG NLSRQAFLLR GSNNYVYNSN
 901 CELFGHYAME LRGSSRNYNV DVGTKLRF*

A predicted signal peptide is highlighted.

The cp6733 nucleotide sequence <SEQ ID 116> is:

```

5      1  ATGAAGACTT CGATTCCCTTG GGTTTTAGTT TCCTCCGTGT TAGCTTTCTC
      51  ATGTCACCTA CAGTCACTAG CTAACGAGGA ACTTTTATCA CCTGATGATA
     101  GCTTTAATGG AAATATCGAT TCAGGAACGT TTAATCCAAA AACTTCAGCC
     151  ACAACATATT CTCTAACAGG AGATGTCTTC TTTTACGAGC CTGGAAGAGG
     201  CACTCCCTTA TCTGACAGTT GTTTTAAGCA AACCACGGAC AATCTTACCT
10    251  TCTTGGGGAA CGGTCAATAGC TTAACGTTTG GCTTTATAGA TGCTGGCATT
     301  CATGCAGGTG CTGCTGCATC TACAACAGCA AATAAGAATC TTACCTTCTC
     351  AGGGTTTTCC TTAATGAGTT TTGATTCCTC TCCTAGCACA ACGGTTACTA
     401  CAGGTGAGGG AACGCTTTCC TCAGCAGGAG GCGTAAATTT AGAAAAATATT
     451  CGTAAACTTG TAGTTGCTGG GAATTTTCTT ACTGCAGATG GTGGAGCTAT
15    501  CAAAGGAGCG TCTTTCCCTT TAACTGGCAC TTCTGGAGAT GCTCTTTTAA
     551  GTAACAATCT TTCATCAACA AAGGGAGGAG CAATTGCTAC TACAGCAGGC
     601  GCTCGCATAG CAAATAACAC AGGTTATGTT AGATTCCAT TAAACATAGC
     651  GTCTACGTCA GGAGGCGCTA TCGATGATGA AGGCACGTCG ATACTATCGA
     701  ACAACAAATT TCTATATTTT GAAGGGAATG CAGCGAAAAC TACTGGCGGT
20    751  GCGATCTGCA ACACCAAGGC GAGTGGATCT CCTGAACTGA TAATCTCTAA
     801  CAATAAGACT CTGATCTTTG CTTCAAACGT AGCAGAAACA AGCGGTGGCG
     851  CCATCCATGC TAAAAAGCTA GCCCTTTCC TGGAGGCTT TACAGAGTTT
     901  CTACGAAATA ATGTCTCATC AGCAACTCCT AAGGGGGGTG CTATCAGCAT
     951  CGATGCCTCA GGAGAGCTCA GTCTTCTGTC AGAGACAGGA AACATTACCT
25   1001  TTGTAAGAAA TACCCCTTACA ACAACCGGAA GTACCGATAC TCCTAAACGT
     1051  AATGCCATCA ACATAGGAAG TAAACGGAAA TTCACGGAAT TACGGGCTGC
     1101  TAAAAATCAT ACAATTTTCT TCTATGATCC CATCACTTCA GAAGGAACCT
     1151  CATCAGACGT ATTGAAGATA AATAACGGCT CTGCGGGAGC TCTCAATCCA
     1201  TATCAAGGAA CGATTCTATT TTCTGGAGAA ACCCTAACAG CAGATGAAC
30   1251  TAAAGTTGCT GACAAATTTAA AATCTTCATT CACGCAGCCA GTCTCCCTAT
     1301  CCGGAGGAAA GTTATTGCTA CAAAAGGGAG TCACTTTAGA GAGCACGAGC
     1351  TCTCTCAAG AGGCCGGTTC TCTCCTCGGC ATGGATTTCAG GAACGACATT
     1401  ATCAACTACA GCTGGGAGTA TTACAATCAC GAACCTAGGA ATCAATGTTG
     1451  ACTCCTTAGG TCTTAAGCAG CCCGTCAGCC TAACAGCAA AGGTGCTTCA
35   1501  AATAAAGTGA TCGTATCTGG GAAGCTCAAC CTGATTGATA TTGAAGGGAA
     1551  CATTATGATA AGTCATATGT TCAGCCATGA CCAGCTCTTC TCTCTATTAA
     1601  AAATCACGGT TGATGCTGAT GTTGATACTA ACGTTGACAT CAGCAGCCTT
     1651  ATCCCTGTTC CTGCTGAGGA TCCTAATTCA GAATACGGAT TCCAAGGACA
     1701  ATGGAATGTT AATTGGAATA CGGATACAGC TACAAATACA AAAGAGGCCA
40   1751  CGGCAACTTG GACCAAAACA GGATTTGTTT CCAGCCCCGA AAGAAAATCT
     1801  GCGTTTAGTAT GCAATACCCCT ATGGGGAGTC TTTACTGACA TTCGCTCTCT
     1851  GCAACAGCTT GTAGAGATCG GCGCAACTGG TATGGAACAC AAACAAGGTT
     1901  TCTGGGTTTC CTCATGACG AACTTCCTGC ATAAGACTGG AGATGAAAAT
     1951  CGCAAAGGCT TCCGTATAC CTCTGGAGGC TACGTCATCG GTGGAAGTGC
45   2001  TCACACTCCT AAAGACGACC TATTTACCTT TCGGTTCTGC CATCTCTTTG
     2051  CTAGAGACAA AGATTGTTTT ATCGCTCACA ACAACTCTAG AACCTACGGT
     2101  GGAATTTTAT TCTTCAAGCA CTCTCATACC CTACAACCCC AAAACTATTT
     2151  GAGATTAGGA AGAGCAAAGT TTTCTGAATC AGCTATAGAA AAATTCCTTA
     2201  GGGAAATTCC CCTAGCCTTG GATGTCCAAG TTTGTTTCAG CCATTTCAGC
50   2251  AACCCTATGG AAACGCACTA TACCTCATTG CCAGAATCCG AAGGTTCTTG
     2301  GAGCAACGAG TGTATAGCTG GTGGTATCGG CCTAGACCTT CCTTTTGTTC
     2351  TTTCCAACCC ACATCCTCTT TTCAAGACCT TCATTCCACA GATGAAAGTC
     2401  GAAATGGTTT ATGTATCACA AAATAGCTTC TTCGAAAGCT CTAGTGATGG
     2451  CCGTGGTTTT AGTATTGGAA GGCTGCTTAA CCTCTCGATT CCTGTGGGTG
55   2501  CGAAATTCGT GCAGGGGGAT ATCGGAGATT CCTACACCTA TGATCTCTCA
     2551  GGATTCCTTG TTTCCGATGT CTATCGTAAC AATCCCCAAT CTACAGCGAC
     2601  TCTTGTGATG AGCCCAGACT CTGGAAGAA TCGCGGTGGC AATCTTTCAA
     2651  GACAGGCATT TTTACTGAGG GGTAGCAACA ACTACGCTA CAACTCCAAT
     2701  TGTGAGCTCT TCGGACATTA CGCTATGGAA CTCGTTGAT CTTCAGGAA
60   2751  CTACAAATGA GATGTTGGTA CCAAACTCCG ATTCTAG
  
```

The PSORT algorithm predicts an outer membrane location (0.924).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 58A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 58B) and for FACS (Figure 58C) analyses. A GST-fusion protein was also expressed.

The cp6733 protein was also identified in the 2D-PAGE experiment (Cpn0451).

- 5 These experiments show that cp6733 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 59

The following *C.pneumoniae* protein (PID 4376814) was expressed <SEQ ID 117; cp6814>:

```

10      1  MHDALLSILA IQELDIKMIR LMRVKEHQK ELAKVQSLKS DIRRKVQEK
      51  LEMENLKTQI RDGENRIQEI SEQINKLENQ QAAVKKMEF NALTQEMTTA
      101  NKERRSLEHQ LSDLMDKQAG GEDLIVSLKE SLASTENSSS VIEKEIFESI
      151  KKINEEGKAL LEQRTKLKHA TNPELLSIYE RLLNNKKDRV VVPIENRVCS
      201  GCHIVLTPQH ENLVRKKDRL IFCEHCSRIL YWQBSQVNAQ ENSTAKRRRR
      251  RAAV*
```

- 15 The cp6814 nucleotide sequence <SEQ ID 118> is:

```

      1  ATGCATGACG CACTTCTAAG CATTTTGGCT ATTCAAGAGC TTGATATTAA
      51  AATGATTGCG CTTATGCGCG TAAAGAAAGA ACATCAGAAA GAATTGGCTA
      101  AAGTCCAATC TTTAAAAAGT GATATTCGTA GAAAAGTTCA GGAAAAAGAA
      151  CTCGAAATGG AGAATTTGAA AACTCAAATT CGAGATGGAG AGAATCGCAT
      201  CCAAGAGATT TCTGAACAAA TCAATAAATT AGAAAATCAG CAAGCTGCTG
      251  TAAAAAAAAT GGATGAGTTT AACGCTCTTA CCCAAGAAAT GACTACAGCA
      301  AACAAAGAAC GTCGCTCTTT AGAGCACCAG CTTAGCGATC TCATGGATAA
      351  GCAAGCTGGA GCGGAAGACC TTATTGCTCT TCTAAAAGAA AGCTTAGCTT
      401  CTACAGAAAA TAGTAGCAGT GTCATTGAAA AAGAAATTTT TGAAAGCATC
      451  AAAAAGATTA ATGAAGAAGG CAAAGCTTTG CTTGAACAAC GGACAGAGTT
      501  AAAGCATGCG ACGAATCCCG AACTACTCAG CATCTATGAG CGTCTATTAA
      551  ACAATAAAAA AGATCGCGTT GTTGTTCCTA TTGAAAATCG TGCTTCGAGT
      601  GGTGTGCATA TTGTCTAAC TCCTCAACAC GAAAATCTTG TAAGAAAGAA
      651  AGACCGACTC ATTTTTTGCG AACATFGCTC TCGAATCTCT TATTGGCAAG
      701  AATCCCAAGT CAATGCTCAG GAAAATTCCA CAGCAAAACG TCGTCGTCGT
      751  CGCGCAGCTG TATAA
```

The PSORT algorithm predicts an inner membrane location (0.070).

- 35 The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 59A) or his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in Western blot (Figure 59B) and FACS (Figure 59C) analyses.

These experiments show that cp6814 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 60

The following *C.pneumoniae* protein (PID 4376830) was expressed <SEQ ID 119; cp6830>:

```

40      1  MKWLPAVAF AAVLPALTAF GDPASVEIST SHTGSGDPTS DAALTGFTQS
      51  STETDGTITY IVGDIITFTF TNIPVPVVT DANDSSSNSS KGGSSSSGAT
      101  SLIRSSNLHS DFDFTKDSVL DLYHLFFPSA SNTLNPALLS SSSSGGSSSS
      151  SSSSSGSAS AVVAADPKGG AAFYSNEANG TLFTTDSGN PGSLTLQNLK
      201  MTGDGAATYS KGPLVFTGLK NLFTGNEISQ KSGGAAYTEG ALTTQAIVEA
      45  251  VTFTGNTSAG QGGAIYVKEA TLFNALDSLK FEKNTSGQAG GGIYTESTLT
      301  ISNITKSIEF ISNKASVPAP APEPTSPAPS SLINSTTIDT STLQTRAASA
      351  TPAVAFVAAV TPTPISTQET AGNGGAIYAK QGISISTFKD LTFKSNSASV
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401	DATLTVDSSST	IGESGGAIFA	ADSIQIQQCT	GTTLFSGNTA	NKSGGGIYAV
451	GQVTLIEDIAN	LKMTNNTCKG	EGGAIYTKKA	LTINNGAILT	TFSGNTSTDN
501	GGAIFAVGGI	TLSDLVEVRF	SKNKTGNYS	PITKAASNTA	PVVSSSTTAA
551	SPAVPAAAAA	PVTNAAKGG	LYSTEGTLVS	GITSILSFEN	NECQNQGGGA
601	YVTKTFQCS	SHRLQFTSNK	AADEGGGLYC	GDDVTLTNLT	GKTLFQENSS
651	EKHGGGLSLA	SGKSLTMTSL	ESFCLNANTA	KENGGGANVP	ENIVLTFTYT
701	PTPNEPAPVQ	QPVYGEALVT	GNTATKSGGG	IYTKNAAFSN	LSSVTFDQNT
751	SENGGALLT	QKAADKTDCS	FTYITNVNIT	NNTATGNGGG	IAGGKAHFDR
801	IDNLTVQSNQ	AKKGGGVYLE	DALILEKVIT	GSVSQNTATE	SGGGIYAKDI
851	QLQALPGSPT	ITDNKVETSL	TTSTNLYGGG	IYSSGAVTLT	NISGTFGITG
901	NSVINATATSQ	DADIQGGGIY	ATTSLSINQC	NTPILFSNNS	AATKKTSTTK
951	QIAGGAIFSA	AVTIENNSQP	IIFLNNSAKS	EATTAATAGN	KDSCGGAIAA
1001	NSVTLTNNPE	ITFKGNYAET	GGAIGCIDLT	NGSPPRKVS	ADNGSVLFPD
1051	NSALNRGGAI	YGETIDISRT	GATFIGNSSK	HDGSAICCST	ALTLPNSQL
1101	IFENNKVTET	TATTKASINN	LGAALYGNNE	TSDVTLISLSA	ENGSIFFKNN
1151	LCTATNKYCS	IAGNVKFTAI	EASAGKAISF	YDAVNVSTKE	TNAQELKLINE
1201	KATSTGTILF	SGELHENKSY	IPQKVTFAHG	NLILGKNAEL	SVVSPFQSPG
1251	TTITMGPQSV	LSNHSKEAGG	IAINNVIIDF	SEIVPTKDNA	TVAPPTLKL
1301	STRNADSKDK	IDITGTVTLL	DPNGNLYQNS	YLGEDRDITL	FNIDNSASGA
1351	VTATNVTLQG	NLGAKKGYLG	TWNLDPNSSG	SKILKWTFFD	KYLRWPYIPR
1401	DNHFYINSIW	GAQNSLVTVK	QGILGNMLNN	ARFEDPAFNN	FWASAIGSFL
1451	RKEVSRNSDS	FTYHGRGYTA	AVDAKPRQEF	ILGAAFSQVF	GHAESYHLD
1501	NYKHKSGSHS	TQASLYAGNI	FYFPAIRSRP	ILFQGVATYG	YMQHDTTTY
1551	PSIEEKNMAN	WDSIAWLFDL	RFSVDLKEPQ	PHSTARLTFTY	TEAKYTRIRQ
1601	EKFTELDYDP	RSFSACSYGN	LAIPTGFSVD	GALAWREIIL	YNKVSAAAYLP
1651	VILRNPKAT	YEVLSTKEKG	NVVNVLPTRN	AARAEVSSQI	YLGSWTLYG
1701	TYTIDASMNT	LVQMANGGIR	FVF*		

A predicted signal peptide is highlighted.

The cp6830 nucleotide sequence <SEQ ID 120> is:

30	1	ATGAAGTGGC	TACCAGCTAC	AGCTGTTTTT	GCTGCCGTAC	TCCCCGCACT
	51	AACAGCCTTC	GGAGATCCCC	CGTCTGTTGA	AATAAGTACC	AGCCATACAG
	101	GATCCGGGGA	TCCTACAAGC	GACGCTGCCT	TAACAGGATT	TACACAAAGT
	151	TCCACAGAAA	CTGACGGTAC	TACCTATACC	ATTGTCGGTG	ATATCACCTT
	201	CTCTACTTTT	ACGAATATTC	CTGTTCCCGT	AGTAACTCCA	GACGCCAACG
35	251	ATAGTTCCAG	CAATAGCTCT	AAAGGAGGAA	GTAGCAGTAG	TGGAGCTACA
	301	TCTCTAATCC	GATCCTCAAA	CCTACACTCC	GATTTTGTAT	TTACAAAAGA
	351	TAGCGTGTGA	GACCTCTATC	ACCTTTCTCT	TCCTTCAGCT	TCAAATACTC
	401	TCAATCCTGC	ACTCCTTTCT	TCCAGTAGCA	GCGGTGGATC	CTCGAGCAGC
	451	AGTAGCTCCT	CATCATCTGG	AAGTGCATCT	GCTGTGTTTG	CTGCGGACCC
40	501	AAAAGGAGGC	GCTGCCTTTT	ATAGTAACGA	GGCTAACGGA	ACTTTAACCT
	551	TCACTACAGA	CTCTGGAAAT	CCCGGCTCCC	TGACTCTTCA	GAATCTTAAA
	601	ATGACCGGAG	ATGGAGCCGC	CATCTACTCG	AAGGGTCCTC	TAGTATTTAC
	651	TGGTTTAAAA	AATCTAACCT	TTACAGGAAA	TGAATCTCAG	AAATCTGGAG
	701	GTGCTGCCTA	TACTGAAGGC	GCACTCACAA	CACAAGCAAT	CGTTGAAGCC
45	751	GTAACCTTTA	CTGGCAACAC	CTCGGCAGGG	CAAGGAGGCG	CTATCTATGT
	801	TAAAGAAGCT	ACCCTATTCA	ATGCTCTAGA	CAGCCTCAAA	TTTGAAAAAA
	851	ACACTTCTGG	GCAAGCTGGT	GGTGAATCT	ATACAGAGTC	TACGCTCACA
	901	ATCTCGAACA	TCACAAAATC	TATTGAATTT	ATCTCTAATA	AAGCTTCTGT
	951	CCCTGCCCCC	GCTCTGAGC	CCACCTCTCC	GGCTCCAAGT	AGCTTAATAA
50	1001	ATTCTACAAC	GATCGATACC	TCGACTCTCC	AAACCCGAGC	AGCATCCGCA
	1051	ACTCCAGCAG	TGGCTCCTGT	TGCTGCCGTA	ACTCCAACAC	CAATCTCTAC
	1101	TCAAGAGACC	GCAGGAAATG	GAGGCGCTAT	CTATGCTAAA	CAAGGTATTT
	1151	CGATATCCAC	GTTTAAAGAT	CTGACCTTCA	AGTCTAACTC	TGCATCGGTA
	1201	GATGCCACCC	TTACTGTCTGA	TTCTAGCACT	ATTGGAGAAT	CTGGAGGTGC
55	1251	TATCTTTGCA	GCAGACTCTA	TACAAATCCA	ACAGTGCACG	GGAACCACTT
	1301	TATTCAGTGG	CAATACTGCC	AATAAGTCTG	GTGGGGGTAT	TTACGCTGTA
	1351	GGACAAGTCA	CCCTAGAAGA	TATAGCGAAT	CTGAAGATGA	CCAACAACAC
	1401	CTGTAAAGGT	GAAGGTGGAG	CCATCTACAC	TAAAAGGCT	TTAACTATCA
	1451	ACAACGGTGC	CATTCTCACT	ACATTTTCTG	GAAATACATC	GACAGATAAT
60	1501	TCTGGGGCTA	TTTTTGCTGT	AGGTGGCATC	ACTCTCTCTG	ATCTTGCTAGA
	1551	AGTCCGCTTT	AGTAAAAATA	AGACCGGAAA	TTATTTCCGT	CCTATTACCA
	1601	AAGCGGCTAG	CAACACAGCT	CCTGTAGTTT	CTAGCTCTAC	AACTGCTGCA
	1651	TCTCCTGCGG	TCCCTGCTGC	CGCTGCAGCA	CCTGTTACAA	ACGCAGCAAA
	1701	AGGAGGGGCT	TTATATAGTA	CAGAAGGACT	GACTGTATCT	GGAATCACAT
65	1751	CGATATTGTC	GTTTGAAAAA	AACGAATGCC	AGAATCAAGG	AGGTGGGGCT

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1801 TACGTTACTA AAACCTTCCA GTGTTCGGAT TCTCATCGCC TCCAGTTTAC
1851 TAGTAATAAA GCAGCAGATG AAGGCGGGGG CCTGTATTGT GGTGACGATG
1901 TCACGCTAAC GAACCTGACA GGGAAAACAC TATTTCAGA GAATAGCAGT
1951 GAGAAACATG GAGGTGGGCT CTCTCTCGCC TCAGGAAAAT CTCTGACTAT
2001 GACATCGTTA GAGAGCTTCT GCTTAAATGC AAATACAGCA AAGGAAAACG
2051 GAGGCGGTGC GAATGTCCCT GAAAATATTG TACTCACCTT CACCTATACT
2101 CCCACTCCAA ATGAACCTGC GCCTGTGCAG CAGCCCGTGT ATGGAGAAGC
2151 TCTTGTACTT GGAATACAG CCACAAAAG TGGTGGGGGC ATTTACACGA
2201 AAAATGCGGC CTTCTCAAAT TTATCTTCTG TAACTTTTGA TCAAAATACC
2251 TCTTCAGAAA ATGGTGGTGC CTTACTTACC CAAAAGCTG CAGATAAAAC
2301 GGACTGTTCT TTCACCTATA TTACAAATGT CAATATCACC AACAATACAG
2351 CTACAGGAAA TGGTGGGGGC ATTGCTGGGG GAAAAGCACA TTTCGATCGC
2401 ATTGATAATC TTACAGTCCA AAGCAACCAA GCAAAGAAAG GTGGTGGGGT
2451 TTATCTTGAA GATGCCCTCA TCCTGGAAAA GGTATTACA GGTCTGTCT
2501 CACAAAATAC AGCTACAGAA AGTGGTGGGG GTATCTACGC TAAGGATATT
2551 CAATACAAG CTCTACCTGG AAGCTTCACA ATTACCGATA ATAAAGTCGA
2601 AACTAGTCTT ACTACTAGCA CTAATTTATA TGGTGGGGGC ATCTATTCCA
2651 GTGGAGCTGT CACGCTAACC AATATATCTG GAACCTTTGG CATTCACGGA
2701 AACTCTGTTA TCAATACAGC GACATCCCAG GATGCAGATA TACAAGGTGG
2751 GGGCATTTAT GCAACCACGT CTCTCTCAAT AAATCAATGT AATACACCCA
2801 TTCTATTTAG CAACAACCTCT GCTGCCACTA AAAAAACATC AACACAAAG
2851 CAAATTGCTG GTGGGGCTAT CTTCTCCGCT GCAGTAACTA TCGAGAATAA
2901 CTCTCAGCCC ATTATTTTCT TAAATAATTC CGCAAAGTCG GAAGCAACTA
2951 CAGCAGCAAC TGCAGGAAAT AAAGATAGCT GTGGAGGAGC CATTCAGCT
3001 AACTCTGTTA CTTTAAACAA TAACCCTGAA ATAACCTTTA AAGGAAATTA
3051 TGCAGAAACT GGAGGAGCGA TTGGCTGTAT TGATCTTACT AATGGCTCAC
3101 CTCCCCTGTA AGTCTCTATT GCAGACAACG GTTCTGTCTT TTTTCAAGAC
3151 AACTCTGCGT TAAATCGCGG AGGCGCTATC TATGGAGAGA CTATCGATAT
3201 CTCCAGGACA GGTGCGACTT TCATCGGTAA CTCTTCAAAA CATGATGGAA
3251 GTGCAATTTG CTGTTCAACA GCCCTAACTC TTGCCCAAA CTCCCAACTT
3301 ATCTTTGAAA ACAATAAGGT TACGGAAACC ACAGCCACTA CAAAAGCTTC
3351 CATAAATAAT TTAGGAGCTG CAATTATGAG AAATAATGAG ACTAGTGACG
3401 TCACTATCTC TTTATCAGCT GAGAATGGAA GTATTTTCTT TAAAAACAAT
3451 CTATGCACAG CAACAAACAA ATACTGCAGT ATTGCTGGAA ACGTAAATTT
3501 TACAGCAATA GAAGCTTCAG CAGGGAAAGC TATATCTTTC TATGATGCAG
3551 TTAACGTTTC CACCAAAGAA ACAATGCTC AAGAGCTAAA ATTAATGAA
3601 AAAGCGACAA GTACAGGAAC GATTCTATTT TCTGGGAAC TTCACGAAA
3651 TAAATCCTAT ATTCCACAGA AAGTCACTTT CGCACATGGG AATCTCATTC
3701 TAGGTAAAAA TGCAGAACTT AGCGTAGTTT CCTTTACCCA ATCTCCAGGC
3751 ACCACAATCA CTATGGGCCC AGGATCGGTT CTTTCCAACC ATAGCAAAGA
3801 AGCAGGAGGA ATCGCTATAA ACAATGTCAT CATTGATTTT AGTGAAATCG
3851 TTCTACTATA AGATAATGCA ACAGTAGCTC CACCCACTCT TAAATTAGTA
3901 TCGAGAACTA ATGCAGATAG TAAAGATAAG ATTGATATTA CAGGAAGTGT
3951 GACTCTTCTA GATCCTAATG GCAACTTATA TCAAAATTC TATCTTGGTG
4001 AAGACCGCGA TATCACTCTT TTCAATATAG ACAATCTGC AAGTGGGGCA
4051 GTTACAGCCA CGAATGTCAC CTTTCAAGGG AATTTAGGAG CTAAAAAAGG
4101 ATATTTAGGA ACCTGGAATT TGGATCCAAA TTCTCGGGT TCAAAAATTA
4151 TTCTAAAATG GACCTTTGAC AAATACCTGC GCTGGCCCTA CATCCCTAGA
4201 GACAACCAC TCTACATCAA CTCTATTTGG GGAGCAGAAA ACTCTTTAGT
4251 GACTGTGAAA CAAGGGATCT TAGGGAACAT GTTGAACAAT GCAAGGTTTG
4301 AAGATCCTGC TTTCAACAAC TTCTGGGCTT CGGCTATAGG ATCTTTCTTT
4351 AGGAAAGAAG TATCTCGAAA TTCTGACTCA TTCACCTATC ATGGCAGAGG
4401 CTATACCGCT GCTGTGGATG CCAAACCTCG CCAAGAATTT ATTTTAGGAG
4451 CTGCCCTTCA TCAGGTTTTT GGTACGCGCG AGTCTGAATA TCACCTTGAC
4501 AACTATAAGC ATAAAGGCTC AGGTCACTCT ACACAAGCAT CTCTTTATGC
4551 TGGCAATATC TTCTATTTTC CTGCGATACG GTCTCGGCCT ATTCATTCC
4601 AAGGTGTGGC GACCTATGGT TATATGCAAC ATGACACCAC AACCTACTAT
4651 CCTTCTATTG AAGAAAAAAA TATGGCAAAC TGGGATAGCA TTGCTTGGTT
4701 ATTTGATCTG CGTTTCAGTG TGGATCTTAA AGAACCTCAA CCTCACTCTA
4751 CAGCAAGGCT TACCTTCTAT ACAGAAGCTG AGTATACCAG AATTCCGCCAG
4801 GAGAAATTC AAGAGCTAGA CTATGATCCT AGATCTTTCT CTGCATGCTC
4851 TTATGGAAAC TTAGCAATTC CTACTGGATT CTCTGTAGAC GGAGCATTAG
4901 CTTGGCGTGA GATTATCTA TATAATAAAG TATCAGCTGC GTACCTCCCT
4951 GTGATCTCA GGAATAATCC AAAAGCGACC TATGAAGTTC TCTCTACAAA
5001 AGAAAAGGGC AACGTAGTCA ACGTTCTCCC TACAAGAAAC GCAGCTCGTG
5051 CAGAGGTGAG CTCTCAAATT TATCTTGGAA GTTACTGGAC ACTCTACGGC
5101 ACGTATACTA TTGATGCTTC AATGAATACT TTAGTGCAA TGGCCAACGG
5151 AGGGATCCGG TTTGTATTCT AG

```

The PSORT algorithm predicts an outer membrane location (0.926).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 60A) or his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in Western blot (Figure 60B) and FACS (Figure 60C) analyses.

- 5 The cp6830 protein was also identified in the 2D-PAGE experiment (Cpn0540) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6830 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 61

- 10 The following *C.pneumoniae* protein (PID 4376854) was expressed <SEQ ID 121; cp6854>:

```

1  MSIAIAREQY AAILDMHPKP SIAMFSSEQA RTSWEKROAH PYLYRLLEII
51  WGVVFKFLGL IFFIPLGLFW VLQKICQNF I LLGAGGWIFR PICRDSNLLR
101 QAYAARLFSA SFQDHVSSVR RVCLQYDEVF IDGLELRLPN AKPDRWMLIS
151 NGNSDCLEYR TVLQGEKDWI FRIAESQSN ILIFNYPGVM KSQGNITRNN
15  201 VVKSQYACVR YLRDEPAGPQ ARQIVAYGYS LGASVQAEAL SKEIADGSDS
251 VRWFVVKDRG ARSTGAVAKQ FIGSLGVWLA NLTHWNINSE KRSKDLHCPB
301 LFIYKDSQGL NLIGDGLFKK ETCFAAPFLD PKNLEBCSGK KIPVAQTGLR
351 HDHILSDDVI KEVAGHIQRH PDN*
```

The cp6854 nucleotide sequence <SEQ ID 122> is:

```

20 1  ATGTCAATAG CTATTGCAAG GGAACAATAC GCAGCTATAT TGGATATGCA
51  TCCTAAACCT TCGATCGCCA TGTTTTCTTC GGAGCAGGCG AGAACTTCTT
101 GGGAGAAACG ACAGGCTCAT CCTTACCTTT ATCGTCTTCT TGAGATCATA
151 TGGGGTGTG TGAAATTTCT TCTCGGCTTA ATCTTCTTTA TTCCCTTGGG
20 201 TCTTTTCTGG GTCCCTTCAGA AGATAATGTC GAATTTTATT CTCTTTGGTG
25 251 CAGGAGGGTG GATTTTTFAGA CCCATATGCA GGGACTCTAA TTTATTTGCGA
301 CAAGCTTACG CCGCGCGTCT TTTCTCCGCT TCATTCCAAG ATCATGTCTC
351 CTCTGTGCGA AGGGTTTGCT TACAGTATGA CGAGGTCTTT ATTGACGGAT
401 TGGAGTTACG TCTTCCCAAT GCTAAGCCAG ATCGATGGAT GTTAATCTCC
451 AATGGAAACT CCGATTGCTT AGAGTATAGG ACAGTGCTGC AAGGGGAAAA
30 501 GGAAGTGGTA TTCCGTATTG CTGAAGAGTC TCAATCCAAC ATTTTAAATCT
551 TCAATTACCC AGGAGTCATG AAGAGCCAAG GGAATATAAC AAGAAACAAT
601 GTAGTCAAAT CTTATCAAGC ATGCGTACGC TATCTTAGAG ATGAACCCGC
651 AGGACCTCAG CCGCGTCAAA TCGTTGCTTA TGGCTATTCT TTAGGAGCTA
701 GTGTTCAAGC CGAAGCATT AAGTAAAGAGA TCGCAGACGG AAGTGATAGC
35 751 GTCCTGTGGT TTGTCGTAA AGATCGAGGA GCTCGCTCTA CAGGAGCCGT
801 TGCTAAACAG TTTATTTGAA GTCTAGGAGT TTGGCTGGCG AATCTTACCC
851 ATTGGAATAT TAATTTTGAA AAGAGAAGCA AGGACTTGCA TTGCCAGAA
901 CTCTTTATTT ATGGCAAGGA TTCCCAAGGT AATCTTATCG GGGATGGATT
951 GTTCAAAAAA GAGACGTGCT TCGCAGCACC ATTTTATAGT CCTAAAAACT
40 1001 TGGAAGAGTG TTCAGGGAAG AAAATCCCTG TAGCTCAGAC CCGTCTAAGA
1051 CACGATCATA TCCTTTCCGA TGATGTGATT AAAGAAGTTG CAGGTCATAT
1101 TCAAAGACAT TTCGATAATT A
```

The PSORT algorithm predicts an inner membrane location (0.461).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 61A.

- 45 The recombinant protein was used to immunise mice, whose sera were used in Western blot (Figure 61B) and FACS (Figure 61C) analyses. A his-tagged protein was also expressed.

These experiments show that cp6854 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 62

The following *C.pneumoniae* protein (PID 4377101) was expressed <SEQ ID 123; cp7101>:

```

1 MYSCYSKGIS HNYLLHPMSR LDIFVFDLSI ANQDQNLLEE IFCSEDTVLF
51 KAYRTTALQS PLAANKNLNIA RKVANYILAD NGEIDTVKLV EAIHHLSQCT
101 YPLGPHRHNE AQDREHLLKM LKALKENPKL KESIKTLFVP SYSTIQNLIR
151 HTLALNPQTI LSTIHVRQAA LTALFTYLRQ DVGSCFATAP AILIHQEYPE
201 RFLKDLNDLI SSGKLSRIVN QREIAPVINL SGCIGELFKP LRILDLYPDP
251 LVKLSSSPGL KKAFAANLI ETLGDSEAQI QQLLSHQYLM QKLQNVHETL
301 TANDIIKSTL LHYYQLQEST VRAIFFKEGL FSKEQVAFST QHPRELSBIQ
351 RVYHYLHAYE EAKSAFIHDT QNPLLKAWAY TLATLADASQ PTISNHIRLA
401 LGWKSSEDPHS LVSIVTHFVE EEVENIRILV QQCEQTYHEA RSQLEYIEGR
451 MRNPLNNQDS QILTMHMRF RQELNKALYE WDSAQEKAKK FLHLPEFLLS
501 FYTQKIPLYF RSSYDAFIQE FAHLYANAPA GFRILFTHGR THPNTWSPYI
551 SINEFIRFLS EFFTSTESL LGKHAVINLE KETSRLVHNI TAMLHTDVFO
601 EALLTRILEA YQLPVPPSIL NHLDQLSQTP WVYVSGGTVD TLLLDYFESS
651 EPLTLTEKHP ENPHELAAPY ADALKDLPTG IKSYLEEGSH SLLSSSPTHV
701 FSIIAGSPLF REAWDNDWYS YTWLRDWWVK QHQDFLODTI LPQLSIYAFI
751 ENFCNKYALQ HVVHDFHDFC SDHSLTLPEL YDKGSRFLSS LFTKDKTVAL
801 IYIRLLLYLM VREVFPVSEQ QLPEVLDNVS SYLGISSRIT YEKFRSLIEE
851 TIPKMTLLSS ADLRHIYKGL LMQSYQKIYT BEDTYLRLLT AMRHHNLAYP
901 APLLFADSNW PSYFPGFILN PGTTEIDLWK FNYAGLQGGP LDNIQELFAT
951 SRPWLYANP IDYGMPPPPG YRSRLPKEFF *
```

The cp7101 nucleotide sequence <SEQ ID 124> is:

```

1 ATGTATTCGT GTTACAGCAA AGGAATATCC CATAACTATC TTCTACATCC
25 51 TATGTCACGT TTGGATATTT TTGTTTTCGA TTCTCTGATC GCAAACCAGG
101 ATCAAATCT TCTTGAGGAA ATTTTCTGTT CTGAAGACAC AGTTTTATTT
151 AAAGCCTACC GTACTACGGC TCTACAATCC CCTCTAGCTG CTAAGAACCT
201 AAATATCGCC CGTAAAGTCG CAAATTATAT CTTAGCTGAC AATGGGGAAA
251 TCGATACAGT AAAGCTTGTC GAAGCCATTC ACCATCTCTC ACAATGTACC
301 TATCCTTTAG GGCCTCATCG CCATAATGAA GCTCAAGATC GTGAACACCT
351 CCTTAAATG CTAAGAGCTC TAAAGGAAA TCCTAAATTA AAAGAAAGCA
401 TCATAACTCT CTTTGTCCCT TCATACTCTA CAATCCAAAA CCTAATTCGC
451 CATACTACTAG CATTGAATCC ACAGACAATT CTCTCTACGA TTCATGTGCG
501 TCAAGCAGCA CTCACAGCGC TCTTCACCTA CCTTCGGCAA GATGTAGGTT
35 551 CCTGTTTTCG TACGGCTCCT GCCATTCTCA TTCACCAAGA ATATCCAGAA
601 CGATTCTCTA AAGATCTCAA TGATCTCATT AGCAGTGGCA AACTCTCTAG
651 AATCGTAAAC CAAAGGGAAA TTGCGGTTCC TATAAACCTT TCGGGATGCA
701 TTGGAGAGCT ATTCAAGCCT TTAAGGATTC TAGATCTTTA TCCTGATCCT
751 CTGGTTAAGC TCTCTCATC TCCAGGACTC AAAAAAGCCT TTTCTGCTGC
40 801 CAATCTTATT GAAACTCTTG GGGATTCTGA AGCACAAATC CAACAGTTGC
851 TCTCGCATCA ATATTGATG CAAAAACTAC AAAATGTCCA TGAGACCTTA
901 ACTGCTAACG ACATTATCAA ATCGACACTT CTGCACTACT ATCAGCTCCA
951 AGAAAGTACT GTACGAGCTA TTTCTTCAA AGAAGGGTTG TTCAGCAAAG
1001 AACAAGTGGC ATTCTCGACG CAACACCCCA GAGAGCTCTC AGAAATACAA
45 1051 CGGGTATACC ACTACTTACA TGCCTATGAA GAAGCAAAAT CTGCTTTTAT
1101 CCATGACACT CAAAATCCCT TACTGAAAGC CTGGGAGTAT ACTTTAGCGA
1151 CTCTTGCGGA TGCTAGCCAA CCTACCATCT CAAACCATAT CCGCCTTGCC
1201 TTAGGATGGA AAAGTGAAGA CCCTCACAGT CTTGTATCTC TAGTTACACA
1251 CTTTGTGAA GAGGAAGTAG AAAACATCCG AATTTTAGTC CAACAATGTG
50 1301 AACAGACCTA TCACGAAGCA CGCTCCCAAC TAGAATATAT TGAAGGGCGG
1351 ATGCGCAACC CACTAAATAA TCAAGACAGT CAGATTTTGA CGATGGATCA
1401 CATGCGCTTC CGTCAAGAAC TCAATAAAGC TCTTTATGAG TGGGATAGTG
1451 CTCAAGAAAA GGCAAGAAA TTTCTACATC TTCCTGAATT CTTACTTTCT
1501 TTTCTATACAA AGCAAAATTC CTTATACTTT CGTAGTTCTT ACGATGCCTT
55 1551 CATTCAAGAA TTTGCTCATC TCTATGCTAA TGCTCCCGCT GGCTTCCGTA
1601 TTTCTTTTAC GCATGGACGC ACCCATCCGA ACACATGGTC CCCCATCTAT
1651 TCGATTATAG AATTATACG TTTTCTTTCT GAATTTCTCA CCTCCACAGA
1701 GTCAGAACTT CTGGGGAAAC ATGCCGTGAT CAATTTAGAG AAAGAAACAT
1751 CTCGGCTCGT CCACAACATC ACTGCCATGC TACACACGGA TGTTTTCCAA
60 1801 GAAGCTCTCC TTACAAGAA TTTAGAAGCC TATCAGCTTC CTGTGCCTCC
1851 CTCCATCTTA AACCACCTAG ATCAGCTGTC ACAAACCTCC TGGGTTTATG
1901 TTTCTGGAGG AACAGTGGAC ACTCTTCTTT TGGATTATTT TGAAAGCTCA
1951 GAACCTCTGA CACTTACAGA AAAGCATCCT GAAAATCCTC ATGAGCTTGC
2001 AGCTTTCTAC GCAGACGCCC TTAAAGATCT CCCTACAGGA ATTAAAGT
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-103-

2051 ATCTAGAAGA AGGATCCCAC TCTCTACTTA GCTCATCACC CACCCACGTT
 2101 TTCTCTATAA TCGCAGGATC TCCTTTATTT CGGGAAGCTT GGGATAATGA
 2151 TTGGTACAGC TATACCTGGC TTCGTGATGT CTGGGTGAAA CAACACCAAG
 2201 ATTTCCCTTCA AGATACTATA TTACCTCAGC TAAGTATCTA TGCTTTCATA
 5 2251 GAGAATTTT GTACAAATA TGCTTTGCAA CATGTAGTTC ATGACTTTCA
 2301 TGATTCTGCT TCCGACCACT CCTTGACTCT TCCGGAGCTC TATGACAAAG
 2351 GATCGCGTTT TCTAAGCTCC TTATTCACCA AAGATAAGAC CGTAGCTCTT
 2401 ATCTATATAC GCCGTCTTCT CTACCTTATG GTCCGTGAAG TCCCTTATGT
 2451 TTCAGAACAA CAGCTTCCAG AAGTCTTAGA TAACGTCTCT TCATATCTCG
 10 2501 GGATTTCCTC TCGTATTACC TATGAGAAAT TCCGCTCCCT GATAGAGGAA
 2551 ACCATCCCTA AAATGACCTT ACTCTCCTCA GCAGACCTGA GGCATATCTA
 2601 TAAAGGTCTC CTCATGCAAA GTTATCAAAA GATCTACACC GAAGAAGATA
 2651 CGTACCTCCG CCTCACCACG GCAATGAGGC ATCATAATCT TGCCATATCCC
 2701 GCTCCTTTGC TCTTTGCAGA CAGTAACTGG CCTTCTATTT ATTTTGGATT
 15 2751 CATCTTAAAT CCAGGAACCA CAGAGATCGA TCTTTGGAAA TTAACTATG
 2801 CAGGGCTGCA AGGACAGCCT CTTGACAATA TCCAGGAGCT GTTCGCAACG
 2851 TCAAGACCCT GGACCCTCTA TGCAATCCT ATAGATTATG GCATGCCACC
 2901 GCCTCCAGGC TACCGCAGCC GCCTCCCTAA AGAATTTTTC TAG

The PSORT algorithm predicts a cytoplasmic location (0.206).

20 The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 62A) or his-tagged product. The proteins were used to immunise mice, whose sera were used in Western blot (Figure 62B) and FACS (Figure 62C) analyses.

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

25 These experiments show that cp7101 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 63

The following *C.pneumoniae* protein (PID 4377107) was expressed <SEQ ID 125; cp7107>:

30 1 MSIVRNSALP LPCLSRSETF KVRSHMKFM KVLTPWIYRK DLWVTAFLLT
 51 AIPGSFAHTL VDIAGEPRHA AQATGVSGDG KIVIGMKVPD DFFAITVGFQ
 101 YIDGHLQPLE AVRPQCSVYP NGITPDGTVI VGTNYAIGMG SVAVKWVNGK
 151 VSELPMLPDT LDSVASAVSA DGRVIGGNRN INLGASVAVK WEDDVITQLP
 201 SLPDAMNACV NGISSDGSII VGTMDVSWR NTAVQWIGDQ LSVIGTLGGT
 251 TSVASAISTD GTVIVGGSSEN ADSQTHAYAY KNGVMSDIGT LGGFYSLAHA
 35 301 VSSDGSVIVG VSTNSEHRYH AFQYADGQMV DLGTLGGPES YAQGVSGDGK
 351 VIVGRAQVPS GDWHAFPCPF QAPSPAPVHG GSTVVTSQNP RGMVDINATY
 401 SSLKNSQQQL QRLLIQHSAK VESVSSGAPS FTSVRGALSK QSPAVQNDVQ
 451 KGTFLSYRSQ VHGNVQNOQL LTGAFMDWKL ASAPKCGFKV ALHYGSQDAL
 501 VERAALPYTE QGLGSSVLSG FGGQVQGRYD FNLGETVVLQ PFMGIQVLHL
 40 551 SREGYSEKNV RFPVSYDSVA YSAATSFMGA HVFASLSPKM STAATLGVER
 601 DLNSHIDEFK GSVSAMGNFV LENSTVSVLR PFASLAMYD VRQQQLVLTLS
 651 VVMNQQLTG TSLVLSQSSY NLSF*

The cp7107 nucleotide sequence <SEQ ID 126> is:

45 1 ATGAGTATAG TCAGAAATTC TGCATTGCCA CTTCCGTGTT TAAGCAGATC
 51 CGAAACCTTT AAAAAAGTTA GGTGCGCATAT GAAATTTATG AAAGTCCTTA
 101 CTCCATGGAT TTATCGAAAA GATCTTTGGG TAACAGCATT CTTACTGACA
 151 GCAATTCAG GATCTTTTGC ACATACTCTT GTTGATATAG CAGGAGAACC
 201 TCGGCATGCT GCTCAAGCAA CAGGAGTTTC TGGAGATGGT AAAATTGTTA
 251 TAGGAATGAA AGTTCCGGAT GATCCTTTTG CTATAACTGT AGGATTTCAA
 50 301 TATATTGATG GGCATTTGCA ACCCTTAGAG GCAGTACGTC CTCAATGCTC
 351 TGTATACCCT AATGGTATAA CCCCAGACGG AACGGTTATT GTGGGTACAA
 401 ACTATGCCAT CGGGATGGGT AGTGTGTGCTG TGAAATGGGT AAATGGCAAG
 451 GTTCTGAAC TTCCCATGCT CCTGACACC CTCGATTCTG TAGCATCGGC
 501 AGTTTCTGCA GATGGAAGAG TGATTGGAGG GAATAGAAAT ATAAATCTTG
 55 551 GCGCTTCTGT TGCTGTGAAA TGGGAGGACG ACGTGATTAC ACAACTTCCT
 601 TCTCTTCTG ATGCTATGAA TGCTTGTGTT AACGGAATTT CTTCAGATGG

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5 651 TTCTATAATT GTAGGAACCA TGGTAGACGT GTCATGGAGA AATACCGCAG
 701 TACAATGGAT CGGGGATCAG CTCTCTGTTA TTGGGACTTT AGGAGGAACT
 751 ACTTCTGTFTG CTAGTGCAAT CTCAACAGAT GGCACGTGTA TTGTAGGAGG
 801 TTCTGAAAAT GCAGATTCTC AGACTCATGC CTATGCTTAT AAAAACGGTG
 851 TTATGAGCGA TATAGGGACC CTCGAGGTT TTTATTCCTT AGCACATGCA
 901 GTATCTTCAG ATGGTTCTGT GATTGTAGGA GTATCCACGA ACTCTGAGCA
 951 TAGATATCAT GCATFCCAAT ATGCTGATGG ACAGATGGTA GATTTAGGAA
 1001 CTTTAGGAGG GCCTGAATCT TATGCTCAAG GTGTGTCTGG AGATGGAAAG
 1051 GTAATTGTGG GTAGAGCACA AGTACCATCT GGAGATTGGC ATGCGTTCCT
 1101 ATGTCCTTTC CAAGCTCCGA GCCCTGCTCC TGTCCATGGG GGAAGCACTG
 1151 TCGTAACTAG CCAGAATCCA CGTGAATGG TAGATATCAA TGCTACGTAC
 1201 TCCTCTTTGA AAAATAGCCA ACAACAATA CAAAGATTGC TTATCCAGCA
 1251 TAGTGCAAAA GTTGAAAGTG TATCCTCAGG AGCACCATCT TTTACAAGTG
 1301 TGAAGAGTGC GATCTCAAAA CAGAGCCCTG CAGTGCAAAA TGATGTACAG
 1351 AAAGGGACGT TTTTAAAGTTA CCGTCCCAA GTTCATGGAA ACGTGCAGAA
 1401 TCAGCAATTG CTCACAGGAG CTTTATGGA CTGGAAACTC GCTTCAGCTC
 1451 CTAAATGCGG CTTTAAAGTA GCTCTCCACT ATGGCTCTCA AGATGCTCTC
 1501 GTAGAACGTG CAGCTCTTCC TTACACAGAA CAAGGCTTAG GAAGCAGTGT
 1551 CTTTGTCAAGT TTTGGAGGAC AAGTTC AAGG ACGCTATGAC TTTAATTTAG
 20 1601 GAGAAACTGT TGTCTGCAA CCTTTATGG GCATTCAAGT TCTCCACCTA
 1651 AGTAGAGAAG GGTATCTCTGA GAAGAATGTT CGATTTCCTG TAAGCTATGA
 1701 TTCTGTAGCC TACTCAGCAG CTAAGCTT TATGGGTGCG CATGTATTTG
 1751 CCTCCCTAAG CCTTAAATG AGTACAGCAG CAACTTTAGG TGTGGAGAGA
 1801 GATCTGAATT CACATATAGA TGAATTTAAG GGATCCGTCT CTGCTATGGG
 25 1851 AAACTTTGTG TTGAAAAT CTACAGTGAG TGTTTTAAAGA CCTTTTGCTT
 1901 CTCTTGCTAT GTACTATGAC GTAAGACAAC AGCAACTCGT GACGTTGTCA
 1951 GTAGTTATGA ATCAACAACC CTTAACAGGC ACACCTAAGCT TAGTAAGCCA
 2001 AAGTAGCTAT AATCTTAGCT TCTAA

The PSORT algorithm predicts an inner membrane location (0.100).

30 The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 63A) or his-tagged product. The proteins were used to immunise mice, whose sera were used in Western blot (Figure 63B) and FACS (Figure 63C) analyses.

These experiments show that cp7107 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

35 Example 64

The following *C.pneumoniae* protein (PID 4376467) was expressed <SEQ ID 127; cp6467>:

40 1 MLRFFAVFIS TLWLITSGCS PSQSSKGIFV VNMKEMPSL DPGKTRLIAD
 51 QTLMRHLYEG LVEEHSQNGE IKPALAESYT ISEDGTRYTF KIKNILWSNG
 101 DPLTAQDFVS SWKEILKEDA SSVLYAFLP IKNARAIPDD TESPENLGVR
 151 ALDKRHLEIQ LETPCAHLFL FLTLPIFFPV HETLRNYSTS FEEMPITCGA
 201 FRPVSLEKGL RLHLEKNPMY HNKSrvKLHK IIVQFISNAN TAAILFKHKK
 251 LDWQGPWPGE PIPPEISASL HQDDQLFSLP GASTWLLFN IQKKPWNNAK
 301 LRKALSLAID KDMLTKVVYQ GLAEPTDHIL HPRLYPGTYP ERKRQNERIL
 351 BAQQLFEEAL DELQMTREDL EKETLTFSTF SFSYGRICQM LRQWKKVLK
 45 401 FTIPIVGQEF FTIQKNFLEG NYSLVFNQWT AAFIDPMSYL MIFANPGGIS
 451 PYHLQDSHFQ TLLIKITQEH KKHLRNQLII BALDYLEHCH ILEPLCHPNL
 501 RIALNKNIKN FNLFVRRTSD FRFIEKL*

A predicted signal peptide is highlighted.

The cp6467 nucleotide sequence <SEQ ID 128> is:

50 1 ATGCTCCGTT TCTTCGCTGT ATTTATATCA ACTCTTTGGC TCATTACCTC
 51 AGGATGTTCC CCATCCCAAT CCTCTAAAGG AATTTTGTG GTAAATATGA
 101 AGGAAATGCC ACGCTCCTTG GATCCTGGAA AAACCTCGTCT CATTGCAGAC
 151 CAAACTCTAA TGGCTCATCT ATATGAAGGA CTCGTCGAAG AACATTCCCA
 201 AAATGGAGAG ATTAAACCAG CCCTTGCAGA AAGCTACACC ATCTCCGAAG
 55 251 ACGGGACTCG GTACACATTT AAAATCAAAA ACATCCTTTG GAGTAACGGA
 301 GACCCTCTGA CAGCTCAAGA CTTTGTCTCC TCTTGAAGG AAATCCTAAA

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351 GGAAGATGCG TCCTCCGTAT ATCTCTATGC GTTTTACCT ATCAAAAATG
 401 CTCGGGCAAT CTTTGATGAT ACTGAGTCTC CAGAAAATCT AGGAGTCCGA
 451 GCTTTAGATA AGCGTCATCT CGAAATTCAG TTAGAAACTC CCTGCGCGCA
 501 TTTCCTACAT TTCTTGACTC TTCTTATTTT TTCCCTGTGT CATGAAACTC
 551 TGCGAAACTA TAGCACCTCT TTTGAAGAGA TGCCCATTAC CTGCGGTGCT
 601 TTCCGCCCTG TGTCTCTAGA AAAAGGCCTG AGACTCCATC TAGAGAAAAA
 651 CCCTATGTAC CATAATAAAA GCCGTGTGAA ACTACATAAA ATTATTGTAC
 701 AGTTTATCTC AAACGCTAAC ACTGCAGCCA TTCTATTCAA ACATAAGAAA
 751 TTAGATTGGC AAGGACCTCC TTGGGGAGAA CCTATCCCTC CAGAAATCTC
 801 AGCTTCTCTA CATCAAGATG ACCAGCTCTT TTCTCTTCCG GCGCTTCGA
 851 CTACATGGTT ACTCTTTAAT ATACAAAAAA AACCTTGGAA CAATGCTAAA
 901 TTACGCAAGG CATTTAGCCT TGCAATAGAC AAAGATATGT TAACCAAAGT
 951 GGTATACCAA GGTCTTGCAG AACCTACAGA TCATATCCTA CATCCAAGAC
 1001 TTTATCCAGG GACCTATCCC GAACGAAAAA GACAAAACGA AAGAAATCTT
 1051 GAGGCTCAAC AACTCTTTGA AGAAGCTCTA GACGAACTTC AAATGACACG
 1101 CGAAGATCTA GAAAAGGAAA CTTTGACTTT CTCAACCTTT TCTTTTCTT
 1151 ACGGAAGGAT TTGCCAAATG CTAAGAGAAC AATGGAAGAA AGTCTTAAAA
 1201 TTTACTATCC CTATAGTAGG CCAAGAGTTT TTCACAATAC AAAAAAATTT
 1251 CCTAGAGGGG AACTATTCCC TAACCGTGAA CCAATGGACC GCAGCATTTA
 1301 TTGATCCGAT GTCTTATCTC ATGATCTTTG CCAATCCTGG AGGAATTTCC
 1351 CCCTATCACC TCCAAGATTC ACATTTTCAA ACTCTTCTCA TAAAGATCAC
 1401 TCAAGAACAT AAAAAACACC TACGAAATCA GCTTATATT GAAGCCCTTG
 1451 ACTATTTAGA ACACTGTCAC ATTCTCGAAC CACTATGTCA TCCAAATCTT
 1501 CGAATTGCTT TGAACAAAAA CATTAATAAC TTTAATCTTT TTGTTGACG
 1551 AACTTCAGAC TTTCTTTTAA TAGAAAAACT ATAG

The PSORT algorithm predicts an outer membrane lipoprotein (0.790).

The protein was expressed in *E.coli* and purified as a his-tag product and a GST-fusion protein, as shown in Figure 64A. The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 64B). The recombinant GST-fusion protein was also used to immunise mice, whose sera were used in a Western blot (Figure 64C) and for FACS analysis (Figure 64D).

These experiments show that cp6467 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 65

The following *C.pneumoniae* protein (PID 4376679) was expressed <SEQ ID 129; cp6679>:

1 MRKMLVLLAS LGLLSPTLSS CTHLGSSGSY HPKLYTSGSK TKGVIAMLPV
 51 FHRPGKSLEP LPWNLQGEFT KEISKRFYAS EKVFLIKHNA SPQTVSQFYA
 101 PIANRLPETI IEQFLPAEFI VATELLEQKT GKEAGVDSVT ASVRVRVFDI
 151 RHHKIALIYQ EIIECSQPLT TLVNDYHRYG WNSKHFDSTP MGLMHSRLFR
 201 EVVARVEGYV CANYS*

A predicted signal peptide is highlighted.

The cp6679 nucleotide sequence <SEQ ID 130> is:

1 ATGCGAAAAA TGTGCTATT ATTGGCATCT TTAGGACTTC TATCCCCAAC
 51 CCTATCCAGC TGCACCTACT TAGGCTCTTC AGGAAGTTAT CATCCTAAGC
 101 TATACACTTC AGGGAGCAAA ACTAAAGGTG TGATTGCGAT GCTTCTGTGA
 151 TTTCATCGCC CAGGAAAGAG TCTTGAACCT TTACCTTGGA ACCTCCAAGG
 201 AGAATTTACT GAAGAGATCA GCAAAAGGTT TTATGCTTCG GAAAAGGTCT
 251 TCCTGATCAA GCACAATGCT TCACCTCAGA CAGTCTCTCA GTTCTATGCT
 301 CCGATTGCGA ATCGTCTACC CGAAACAATT ATTGAGCAAT TTCTTCCTGC
 351 AGAATTCATT GTTGCTACAG AACTGTTAGA ACAAAGACA GGGAAAGAAG
 401 CAGGTGTCGA TTCTGTAACA GCGTCTGTAC GTGTTCGCGT TTTTGATATC
 451 CGTCATCATA AAATAGCTCT CATTTATCAA GAGATTATCG AATGCAGCCA
 501 GCCTTTAAT ACCCTAGTCA ATGATTATCA TCGCTATGGC TGGAACTCAA
 551 AACATTTTGA TTCAACGCCC ATGGGCTTAA TGCATAGCCG TCTTTTCCGC

601 GAAGTTGTTG CCAGAGTTGA GGGCTATGTT TGTGCTAACT ACTCGTAG

The PSORT algorithm predicts an inner membrane location (0.149).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 65A) and as a GST-fusion product (Figure 65B). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 65C) and for FACS analysis.

These experiments show that cp6679 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 66

The following *C.pneumoniae* protein (PID 4376890) was expressed <SEQ ID 131; cp6890>:

```

10      1  MKQLLFCVCV FAMSCSAYAS PRRQDPSVMK ETRNNYGI VSGQEWVKRG
      51  SDGTTTKVLK NGATLHEVYS GLLHGEITL TFPHTTALDV VQIYDQGRLV
      101  SRKTFPVNGL PSQELFNED GTFVLTRWPD NNSDTITKP YFIETTYQGH
      151  VIEGSYTSFN GKYSSSIHNG EGVRSVFSSN NILLSEETFN EGVMVKYTTF
      201  YPNRDPESIT HYQNGQPHGL RLTYLQGGIP NTIEBWRYGF QDGTITVFKN
      251  GCKTSEIAYV KGVKEGLELR YNBQEIVAEB VSWRNDFLHG ERKIYAGGIQ
      301  KHEWYYRGRS VSKAKFERLN AAG*

```

A predicted signal peptide is highlighted.

The cp6890 nucleotide sequence <SEQ ID 132> is:

```

20      1  ATGAAACAAT TACTTTTCTG TGTTCGCGTA TTTGCTATGT CATGTTCTGC
      51  TTACGCATCC CCACGACGAC AAGATCCTTC TGTATGAAG GAAACATTCC
      101  GAAATAATTA TGGCATTATT GTTCCGGTC AAGAATGGGT AAAGCGTGGT
      151  TCTGACGGCA CCATCACCAA AGTACTCAA AATGGAGCTA CCCTGCATGA
      201  AGTTTATTCT GGAGGCCTCC TTCATGGGGA AATTACCTTA ACGTTTCCCC
      251  ATACCACAGC ATTGGACGTT GTTCAAATCT ATGATCAAGG TAGACTCGTT
      301  TCTCGCAAAA CCTTTTGTGT GAACGGTCTT CCATCTCAAG AAGAGCTGTT
      351  CAATGAAGAT GGCACGTTTG TCCTCACACG ATGGCCGGAC AACACGACA
      401  GTGATACCAT CACAAAGCCT TACTTCATAG AAACGACATA TCAAGGGCAT
      451  GTCATAGAAG GAAGTTATAC TTCCTTTAAT GGGAAATACT CCTCATCCAT
      501  CCACATGGA GAGGGAGTTC GTTCTGTGTT CTCCTCCAAT AACATCCTTC
      551  TTTCTGAAGA GACCTTCAAT GAAGGTGTCA TGGTGAAATA TACCACATTC
      601  TATCCGAATC GCGATCCCGA ATCGATTACT CATTATCAAA ATGGACAGCC
      651  TCACGGCTTA CGGCTAACAT ATCTACAAGG TGGCATCCCC AATACGATAG
      701  AGGAGTGGCG TTATGGCTTT CAAGACGGAA CGACCATCGT ATTTAAAAAT
      751  GGTGTGAAGA CATCTGAGAT CGCTTATGTT AAGGGAGTGA AAGAAGGTTT
      801  AGAAGTGGCG TACAATGAAC AGGAAATTGT AGCTGAAGAA GTTCTCTGGC
      851  GTAATGATTT TCTGCATGGA GAACGTAAGA TCTATGCTGG AGGAATCCAA
      901  AAGCATGAAT GGTATTACCG CGGGAGATCT GTATCTAAAG CCAAATTCGA
      951  GCGGCTAAAT GCTGCAGGAT AG

```

The PSORT algorithm predicts an outer membrane location (0.940).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 66A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 66B) and for FACS analysis. A his-tagged protein was also expressed.

These experiments show that cp6890 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 67

The following *C.pneumoniae* protein (PID 6172323) was expressed <SEQ ID 133; cp0018>:

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1 MKTSVSMLLA LLCSGASSIV LHAATTPPLNP EDGFIGEGNT NTFSPKSTTD
 51 AAGTTYSLTG EVLYIDPGKG GSI TGTCTFVE TAGDLTF LGN GNTLKFLSVD
 101 AGANLAVAHV QGSKNLSFTD FLSLVITESP KSAVTTGKGS LVSLGAVQLQ
 151 DINTLVLTSTN ASVEDGGVIK GNSCLIQGIK NSAI FGQNTS SKKGAISTT
 5 QGLTIENNLG TLKFNENKAV TSGGALDLGA ASTFTANHEL IFSQNKTSGN
 201 AANGGAINCS GDLTFTDNTS LLLQENSTMQ DGGALCSTGT ISITGSDSIN
 251 VIGNTSGQKG GAISAASLKI LGGQGGALFS NNVVTHATPL GGAIFINTGG
 301 SLQLFTQGGD IVFEGNQVTT TAPNATTKRN VIHLESTAKW TGLAASQGNA
 351 IYFYDPITTN DTGASDNLRI NEVSANQKLS GSI VFSGERL STAEIAENL
 401 TSRLNQPVTL VEGSLVLRQG VTLLTQGF SQ EPESTLLLDL GTSL*
 10 451

A predicted signal peptide is highlighted.

The cp0018 nucleotide sequence <SEQ ID 134> is:

1 ATGAAGACTT CAGTTTCTAT GTTGTGGCC CTGCTTTGCT CGGGGGCTAG
 51 CTCTATTGTA CTCCATGCCG CAACCACTCC ACTAAATCCT GAAGATGGGT
 15 101 TTATTGGGGA GGGCAATACA AATACCTTTT CTCCGAAATC TACAACGGAT
 151 GCTGCAGGAA CTACCTACTC TCTCACAGGA GAGGTCTGT ATATAGATCC
 201 GGGGAAAGGT GGTCAATTA CAGGAACTTG CTTTGTAGAA ACTGCTGGCG
 251 ATCTTACATT TTTAGGTAAT GGAAATACCC TAAAGTTCCT GTCGGTAGAT
 301 GCAGGTGCTA ATATCGCGGT TGCTCATGTA CAAGGAAGTA AGAATTTAAG
 20 351 CTTTACAGAT TTCTTTCTC TGGTGATCAC AGAATCTCCA AAATCCGCTG
 401 TTACTACAGG AAAAGGTAGC CTAGTCAGTT TAGGTGCAGT CCAACTGCAA
 451 GATATAAACA CTCTAGTTCT TACAAGCAAT GCCTCTGTCG AAGATGGTGG
 501 CGTGATTAAA GGAAGCTCCT GCTTGATTCA GGGAAATCAA AATAGTGCAG
 551 TTTTGGACA AAATACATCT TCGAAAAAG GAGGGCGAT CTCCACGACT
 25 601 CAAGGACTTA CCATAGAGAA TAACCTAGGG ACGCTAAAGT TCAATGAAAA
 651 CAAAGCAGTG ACCTCAGGAG GCGCCTTAGA TTTAGGAGCC GCGTCTACAT
 701 TCACTGCGAA CCATGAGTTG ATATTTTAC AAAATAAGAC TTCTGGGAAT
 751 GCTGCAAAATG GCGGAGCCAT AAATTGCTCA GGGGACCTTA CATTTACTGA
 801 TAACACTTCT TTGTTACTTC AAGAAAATAG CACAATGCAG GATGGTGGAG
 30 851 CTTTGTGTAG CACAGGAACC ATAAGCATTA CCGGTAGTGA TTCTATCAAT
 901 GTGATAGGAA ATACTTCAGG ACAAAGGA GGAGCGATTT CTGCAGCTTC
 951 TCTCAAGATT TTGGGAGGCG AGGGAGGCGC TCTCTTTCT AATAACGTAG
 1001 TGACTCATGC CACCCTCTA GGAGGTGCCA TTTTATCAA CACAGGAGGA
 1051 TCCTTGCAGC TCTTCACTCA AGGAGGGGAT ATCGTATTCG AGGGGAATCA
 35 1101 GGTCACATA ACAGCTCCAA ATGCTACCAC TAAGAGAAAT GTAATTCACC
 1151 TCGAGAGCAC CGCGAAGTGG ACGGACTTG CTGCAAGTCA AGGTAACGCT
 1201 ATCTATTTCT ATGATCCCAT TACCACCAAC GATACGGGAG CAAGCGATAA
 1251 CTTACGTATC AATGAGGTCA GTGCAAATCA AAAGCTCTCG GGATCTATAG
 1301 TATTTTCTGG AGAGAGATTG TCGACAGCAG AAGCTATAGC TGAAAATCTT
 40 1351 ACTTCGAGGA TCAACCAGCC TGTCACCTTA GTAGAGGGGA GCTTAGTACT
 1401 TAAACAGGGA GTGACCTTGA TCACACAAGG ATTCTCGCAG GAGCCAGAAT
 1451 CCACGCTTCT TTTGGATCTG GGGACCTCAT TATAA

The PSORT algorithm predicts outer membrane (0.935).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 67A). The
 45 recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure
 67B) and for FACS analysis.

These experiments show that cp0018 is a surface-exposed and immunoaccessible protein, and that it
 is a useful immunogen. These properties are not evident from the sequence alone.

Example 68

50 The following *C. pneumoniae* protein (PID 4376262) was expressed <SEQ ID 135; cp6262>:

1 MRKLRLAIV LIALSIILIA GGVLLTVAI PGLSSVISSP AGMGACALGC
 51 VMLALGIDVL LKKREVPIVL ASVTTTPGTG SPRSGISISG ADSTIRSLPT
 101 YLLDEGHPQS MRKLRLAIV LIVFSIILIA SGVLLTVAI PGLSSVISSP
 151 AGMGACALGC VMLALGIDVL LKKREVPIVL ASVTTTPGTG SPRSGISISG
 55 201 ADSTIRSLPT YPLDEGHPQS MRKLRLAIV LIVFSIILIA SGVLLTVAI
 251 PGLSSIISP AEMGACALGC VMLALGIDVL LKKREVPIVL PAPIPEEVI

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301 DDIDEESIRL QQEAEAAALR LPEEMSAFEG YIKVVESHLE NMKSLPYDGH
 351 GLEEKTKHQI RVVRSLSKAM VPEFLDIRRI FREERFFFLS ARKRLIDLAT
 401 TLVERKILTE QLERNNLRKA PSYLYQDSIF KKIIDNFEKL AWKFMILSKS
 451 ICRFTIIFEN HEHGVAKSL L HKNVLLLEKV IYRSLOKSYR DIGMSSAKMK
 501 ILHGNPFFSL EDNKKTIMKE HAEMLESLS YRKVFLALSD ENVVDTPSDP
 551 KKWDLSGIPC RDALSEISRD BQWQKKAHLK HQESLYTQAR DRLTDQSSKE
 601 NQKELEKAEQ EYISSWERVK KFEIERVQER IRAIQKLYPN ILEREBBTTG
 651 QETVTPVQGG TTASSDLTDI LGRIEVSSRE DNQONQESCVK VLRSHVEVMS
 701 WEVKQBYGPK KKEFQDQMG S LERFFTEHIE ELEVLQKDYS KHL SYFKKVN
 751 NKKEVQYAKF RLKLVESDLR GILAQTESAB SLLTQEELPI LATRGALSKA
 801 VFKGSLCCAL ASKAKPYFEE DPRFQSDTQ LRALTRLRQE AKASLEEEIK
 851 RFSNLENDIA KERRLLKESK QTFERAGLG V LRRIAVESTY DLRLSLTNTWE
 901 GTPSEKQYVF SMYLNYYNEE KRRAKTRLVE MTQRYRDFKM ALEAMQFNEE
 951 ALLQBELSIQ APSE*

A predicted signal peptide is highlighted.

The cp6262 nucleotide sequence <SEQ ID 136> is:

1 ATGAGGAAAC TFCGTATTCT TCGATCGTT CTCATAGCTT TGAGCATTAT
 51 TTTGATTGCA GGTGGTGTGG TATTGCTTAC TGTAGCGATC CCTGGATTAA
 101 GTTCAGTCAT TTCTTCCCCG GCAGGATGG GTGCCGTGTC TTTGGGATGT
 151 GTGATGCTTG CTTTAGGGAT CGATGTCTT CTGAAGAAAC GAGAAGTCCC
 201 TATAGTTCTC GCATCTGTAA CTACGACACC AGGAAGTGGC AGCCCTAGAA
 251 GTGGTATTTC TATTTAGGGA GCTGATAGCA CCATACGTTT TCTTCCCTACG
 301 TATCTCTTGG ACGAGGGACA TCCACAATCC ATGAGGAAAC TFCGTATTCT
 351 TCGCATCGTT CTCATAGTTT TTAGCATTAT TTTGATTGCA AGTGGTGTGG
 401 TATTGCTTAC TGTAGCGATC CCTGGATTAA GTTCAGTCAT TTCTTCCCCG
 451 GCAGGATGG GTGCCGTGTC TTTGGGATGT GTGATGCTTG CTTTAGGGAT
 501 CGATGTTCTT CTGAAGAAAC GAGAAGTCCC TATAGTTCTC GCATCTGTAA
 551 CTACGACACC AGGAAGTGGC AGCCCTAGAA GTGGTATTTC TATTTAGGGA
 601 GCTGATAGCA CCATACGTTT TCTTCCCTACG TATCCCTTGG ACGAGGGACA
 651 TCCACAATCC ATGAGGAAAC TFCGTATTCT TCGATCGTT CTCATAGTTT
 701 TTAGCATTAT TTTGATTGCA AGTGGTGTGG TATTGCTTAC TGTAGCGATC
 751 CCTGGATTAA GCTCGATCAT TTCTTCCCCA GCGGATGG GTGCTGTGTC
 801 TTTGGGATGT GTGATGCTTG CTTTGGGGAT CGACGTCTT CTGAAGAAAC
 851 GAGAAGTCCC TATAGTAGTT CCCGCACCTA TTCCTGAAGA AGTCGTCATA
 901 GATGATATAG ATGAAGAGAG TATACGGCTG CAGCAGGAAG CTGAAGCCGC
 951 TTTAGCAAGA CTTCTGAGG AGATGAGTGC ATTTGAAGGT TACATAAAAG
 1001 TTGTCGAGAG TCATTGGAG AACATGAAAA GCCTGCCTTA TGATGGTCAT
 1051 GGGCTAGAAG AGAAAACGAA ACATCAGATA AGAGTCGTCA GATCTTCTTT
 1101 GAAGGCTATG GTTCCAGAA TTTTAGATAT CAGAAGAATT TTTGAAGAAG
 1151 AAGAGTTCTT TTTTCTCTCA GCTCGCAAAC GACTTATAGA TTTAGCTACT
 1201 ACTTTAGTAG AGAGAAAAAT TTTAACAGAG CAACCTGAGC GCAATAATTT
 1251 AAGGAAAGCG TTTTCTTATT TATATCAGGA CTCAATTTTT AAAAAAATTA
 1301 TTGATAACTT CGAGAAGTTA GCATGGAAT TATGATTTT GAGTAAATCA
 1351 ATTTGTCGAT TTACAATTAT TTTGAAAAT CATGAACATG GTGTAGCAAA
 1401 GAGCCTGTTA CACAAGAATG CAGTGTTACT GGAGAAGGTA ATCTATAGGA
 1451 GTTTGCAAAA AAGCTATAGA GATATAGGCA TGTCATCTGC AAAGATGAAA
 1501 ATCTTGCACG GCAACCTTT TTTCTCTTTG GAAGATAATA AAAAGACGAT
 1551 AATGAAAGAA CACGCAGAGA TGCTTGAAAG TCTCAGTAGC TATAGGAAGG
 1601 TATTTTTPAGC TCTATCTGAT GAGAACGTTG TAGATACACC TAGCGATCCA
 1651 AAGAAATGGG ATTTGTGAGG AATCCCTGT AGGGACGCGT TGTCTGAGAT
 1701 TTCTCGTGAT GAACAGTGGC AGAAGAAAGC ACATCTAAAG CATCAAGAGT
 1751 CCCTCTATAC GCAAGCTAGG GATCGTTTAA CAGACCAGAG CTCTAAAGAA
 1801 AATCAGAAAG AGTTAGAGAA AGCTGAACAA GAGTACATAT CTCTTTGGGA
 1851 ACGGGTTAAA AAATTTGAGA TTGAGAGAGT ACAGGAGAGG ATACGGGCAA
 1901 TTCAAAGCT TTATCTTAAT ATCCTCGAGA GAGAAGAAGA AACCACAGGT
 1951 CAGGAGACTG TGA CTCAAC TGTTCAAGGG ACGACGGCTT CATCCGATTT
 2001 AACAGATATT TTAGGAAGAA TAGAGGTCTC CAGTAGGGAG GATAATCAGA
 2051 ATCAAGAGTC TTGTGTAAAA GTCTTAAGAA GTCATGAGGT AGAAATGAGC
 2101 TGGGAAAGTCA AACAAGAGTA TGGCCCTAAG AAAAAAGAA TTTAGGATCA
 2151 AATGGGTTCT TTAGAGAGGT TTTTACAGA GCATATTGAA GAGTTAGAAG
 2201 TATTACAGAA GGA CTACTCT AAACACTGT CTATTTTTAA AAAAGTAAAC
 2251 AATAAGAAAG AGGTTCAATA TGCGAAGTTT AGGTTGAAGG TTTTAGAGTC
 2301 AGATTTAGAA GGGATTCTAG CTCAGACTGA GAGTGCTGAG AGTCTGTTAA
 2351 CTCAAGAGA ACTTCCGATT CTTGCAACTC GGGGAGCCTT AGAGAAAGCT
 2401 GTTTTCAAGG GGAGTCTATG TTGCGCGCTA GCAAGCAAAG CAAAACCCCTA

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2451 TTTTGAAGAG GATCCAGAT TCCAAGATTC TGATACGCAA TTGCGAGCTC
2501 TGACTCTAAG GTTACAGGAG GCTAAGGCAA GCCTGGAAGA AGAGATAAAG
2551 AGATTTTCAA ATCTTGAGAA CGATATTGCA GAGGAAAGAC GCCTTCTTAA
5 2601 AGAGAGCAAG CAGACGTTTCG AAAGAGCAGG TTTAGGGGTT CTCCGAGAAA
2651 TTGCAGTCGA GTCTACTTAT GATTTCGCTT CCTTAACAAA TACATGGGAA
2701 GGGACCCAG AGAGTGAGAA GGTCTATTTT AGCATGTATC TTAATTATTA
2751 CAACGAAGAG AAACGTAGGG CTAAAACAAG ATTGGTTGAA ATGACACAGA
2801 GGTATAGAGA TTTTAAAATG GCCTTGGAAG CTATGCAGTT TAATGAAGAA
2851 GCCCTTTTGC AAGAGGAACT CTCATTCAA GCTCCAGTG AATAA

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10 The PSORT algorithm predicts inner membrane (0.660).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 68A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 68B) and for FACS analysis.

These experiments show that cp6262 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 69

The following *C.pneumoniae* protein (PID 4376269) was expressed <SEQ ID 137; cp6269>:

```

1 MYQENLRLLLE RLLYNSVQKS YADRLFSYRK TKMVHDTPLI PWEEKKEKCA
51 EAERKAFLEQQ KILLDYGKSI FWLNENDEIN LNDPWSWGLN TVRTRKVFQE
20 101 VDDSERWNHK VLIQKLEDDY EKLLLESSKE STEANKKLLS DLVDRLEDAK
151 TKFFFLKKQEE VETRVKDLRA RYGGTVDPKQ DTEAKKKVEL EASLETFLDS
201 IESELVQCLE DQDIYWKEQD VKDLARTQEL EEQDIEAKRE EAAEDLRSLN
251 ERLKRSKTM LDRKWHIENA EDSITWWT SQ IEMKDMKARL KILKEDITSV
301 LPEIDEIETC LSLEELPLLT TRELLTKSYL KFKICSETLL KMTSVFENNI
25 351 YVQYEVQLQ NLGFKLQGIS QRFGKKQDDF ANLEEQVALQ KKRLRELTON
401 FFIQGFNFMK EDFKAAAKDL YIRSTAEQKM NFDVPCMELF RRYHHEVNKP
451 LLELMYNCAD SYRDAKKKLC SLRLDEKELL QKEIKKBEFY QKKQQRHADR
501 SRHTTYQKLR IAEELALELK KKI*

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The cp6269 nucleotide sequence <SEQ ID 138> is:

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30 1 ATGTACCAGG AGAATCTAAG ATTGTTGGAA AGGCTTCTTT ATAATAGTGT
51 TCAAAAGAGC TATGCGGATC GGCTGTTTTT CTATGAAAAG ACAAAGATGG
101 TGCACGATAC TCCGCTGATT CCTTGGGAAG AGGATAAGGA AAAATGTGCT
151 GAAGCTGAGA AAGCTTTCTT AGAGCAACAG AAGATTCTCC TAGATTATGG
201 AAAATCTATC TTTTGGCTGA ATGAGAACGA TGAGATCAAT TTAAACGATC
35 251 CTTGGAGTTG GGGTCTTAAT ACGGTGAGGA CTAGGAAAGT ATTCCAAGAG
301 GTTGACGACA GTGAACGTTG GAATCATAAG GTACTCATTC AAAAAGTCGA
351 GGACGATTAT GAGAACTTTC TAGAGGAAAG TTCAAAAGAG TCTACTGAAG
401 CAAATAAGAA GCTTTTATCT GACTTAGTAG ATCGTCTTGA AGATGCTAAG
451 ACAAATTTT TCCTGAAGAA ACAGGAGGAG GTGGAGACTC GCGTTAAGGA
40 501 TCTTAGAGCT CGATATGGAG GCACAGTAGA TCCTAAGCAG GATACGGAAG
551 CTAAGAAGAA AGTCGAATTG GAGGCTAGCT TAGAAACCTT TTTAGATTCC
601 ATCGAATCAG AGCTAGTACA GTGTTTAGAA GATCAAGATA TATATTGGAA
651 AGAACAGGAT GTCAAAGATC TAGCACGTAC GCAAGAGCTC GAGGAACAAG
701 ATATTGAAGC GAAGAGGGAA GAAGCTGCCG AAGACCTAAG AAGTCTTAAT
45 751 GAGCGTTTAA AGAAGTCAAA AACTATGTTA GATAGGGCTA AATGGCATAT
801 TGAAAATGCT GAGGACAGTA TTACCTGGTG GACTAGTCAG ATAGAAATGA
851 AGGATATGAA AGCAAGACTG AAGATCTTAA AAGAAGATAT AACAAGTGTT
901 CTACCTGAAA TAGATGAGAT TGAAACGTGT TTAAGCTTAG AGGAGCTTCC
951 TTTGCTTACG ACCAGGGAAC TCTTAACTAA GTCCTACCTA AAGTTTAAGA
50 1001 TTTGTTTCGA AACACTATTA AAAATGACTT CTGTGTTTGA GAACAATATC
1051 TATGTTTCAGG AGTACGAGGT TCAGCTGCAA AATCTAGGGT TTAAGTTACA
1101 AGGTATATCT CAGAGATTTCG GAAAGAAACA AGACGATTTT GCGAATCTAG
1151 AGGAACAGGT TGCTTTGCAA AAGAAACGAC TCAGAGAGCT CACTCAGAAT
1201 TTTGAAATAC AAGGATTCAA TTTTATGAAA GAAGATTTTA AGGCAGCCGC
55 1251 TAAAGATCTT TATATAAGAA GTACAGCTGA ACAAAGATG AACTTTGATG
1301 TGCCTTGCAT GGAGCTCTTC CGTAGGTATC ATGAGGAGGT CAACAAGCCG
1351 CTTCTTGAGT TGATGTACAA TTGTGCAGAC AGTTATAGAG ATGCTAAGAA

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1401 AAAGCTTTGC TCTCTACGTC TTGATGAAAA AGAGTTATTA CAAAAAGAAA
1451 TCAAGAAAGA GGAATTTTAT CAAAAGAAAC AACAAAGGCA TGCAGATAGA
1501 TCACGTCATA CTACGTATCA AAAGCTACGA ATTGCTGAAG AGCTTGCTCT
1551 TGAGCTGAAG AAGAAATCT AA

```

5 The PSORT algorithm predicts cytoplasmic location (0.412).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 69A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 69B) and for FACS analysis.

These experiments show that cp6269 is a surface-exposed and immunoaccessible protein, and that it
10 is a useful immunogen. These properties are not evident from the sequence alone.

Example 70

The following *C.pneumoniae* protein (PID 4376270) was expressed <SEQ ID 139; cp6270>:

```

1 MKIPLRFLLI SLVPTLSMSN LLGAATTEEL SASNSFDGTT STTSFSSKTS
51 SATDGTNYVF KDSVVIENVP KTGETQSTSC FKNDAAAGDL NFLGGGFSFT
15 FSNIDATTAS GAAIGSEAN KTVTLSGFSA LSFLKSPAST VTNGLGAINV
151 KGNLSLLDND KVLIQDNFST GDGGAINCAG SLKIANNKSL SFIGNSSSTR
201 GGAHTKNLT LSSGGETLFQ GNTAPTAAGK GGAIAIADSG TLSISGDSGD
251 IIFEGNTIGA TGTVSHSAID LGTSAKITAL RAAQGHTIYF YDPITVTGST
301 SVADALNINS PDTGDNKEYT GTIVFSGEKL TEAEAKDEKN RTSKLLQNV
351 FKNGTVVLKG DVVLSANGFS QDANSKLIMD LGTSLVANTE SIELTNLEIN
401 IDSLRNGKKI KLSAATAQKD IRIDRPVULA ISDESPYQNG FLNEDHSYDG
451 ILELDAGKDI VISADSRSID AVQSPYGYQG KWTINWSTDD KKATVSWAKQ
501 SFNPTEAQEA PLVPNLLWGS FIDVRSFQNF IELGTEGAPY EKRFWVAGIS
551 NVLHRSGREN QRKFRHVSOG AVVGASTRMP GGDTLSLGFA QLFARDKDYF
25 601 MNTNFAKTYA GSLRLQHDAS LYSVVSILLG EGGLREILLP YVSKTLPCSF
651 YGQLSYGHTD HRMKTESLPP PPPTLSTDHT SWGGYVWAGE LGTRVAVENT
701 SGRGFFQEYT PFVKVQAVYA RQDSFVELGA ISRDFSLSHL YNLAIPGK
751 LEKRFABQYY HVVAMYSQDV CRSNPKCTTT LLSNQGSWKT RGSNLRQAG
801 IVQASGFRSL GAAAEFLGNF GFWRGSSRS YNVDAGSKIK F*

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30 A predicted signal peptide is highlighted.

The cp6270 nucleotide sequence <SEQ ID 140> is:

```

1 ATGAAGATTC CACTCCGCTT TTTATTGATA TCATTAGTAC CTACGCTTTC
51 TATGTCGAAT TTATTAGGAG CTGCTACTAC CGAAGAGTTA TCGGCTAGCA
101 ATAGCTTCGA TGGAACTACA TCAACAACAA GCTTTTCTAG TAAACATCA
35 151 TCGGCTACAG ATGGCACCAA TTATGTTTTT AAAGATTCTG TAGTTATAGA
201 AAATGTACCC AAAACAGGGG AAACCTCAGT TACTAGTTGT TTTAAAAATG
251 ACGCTGCAGC TGGAGATCTA AATTTCTTAG GAGGGGATT TCTTTTACA
301 TTTAGCAATA TCGATGCAAC CACGGCTTCT GGAGCTGCTA TTGGAAGTGA
351 AGCAGCTAAT AAGACAGTCA CGTTATCAGG ATTTTCGGCA CTTTCTTTTC
40 401 TTAAATCCCC AGCAAGTACA GTGACTAATG GATTGGGAGC TATCAATGTT
451 AAAGGGAATT TAAGCCTATT GGATAATGAT AAGGTATTGA TTCAGGACAA
501 TTTCTCAACA GGAGATGGCG GAGCAATTAA TTGTGCAGGC TCCTTGAAGA
551 TCGCAAAACA TAAGTCCCTT TCTTTTATTG GAAATAGTTC TTCAACACGT
601 GGCGGAGCGA TTCATACCAA AAACCTCACA CTATCTTCTG GTGGGGAAC
45 651 TCTATTTCAG GGAATACAG CGCCTACGGC TGCTGGTAAA GGAGGTGCTA
701 TCGCGATTGC AGACTCTGGC ACCCTATCCA TTTCTGGAGA CAGTGGCGAC
751 ATTATCTTTG AAGGCAATAC GATAGGAGCT ACAGGAACCG TCTCTCATAG
801 TGCTATTGAT TTAGGAATA GCGCTAAGAT AACTGCGTTA CGTGCTGCGC
851 AAGGACATAC GATATACTTT TATGATCCGA TTACTGTAAC AGGATCGACA
50 901 TCTGTTGCTG ATGCTCTCAA TATTAATAGC CCTGATACTG GAGATAACAA
951 AGAGTATACG GGAACCATAG TCTTTTCTGG AGAGAAGCTC ACGGAGGCAG
1001 AAGCTAAAGA TGAGAAGAAC CGCACTTCTA AATTACTTCA AAATGTTGCT
1051 TTTAAAAATG GGACTGTAGT TTTAAAAGGT GATGTCGTTT TAAGTGCAGAA
1101 CGGTTTCTCT CAGGATGCAA ACTCTAAGTT GATTATGGAT TTAGGGACGT
55 1151 CGTTGGTTGC AAACACCGAA AGTATCGAGT TAACGAATTT GGAATTAAT
1201 ATAGACTCTC TCAGGAACGG GAAAAAGATA AAACCTCAGT CTGCCACAGC

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1251 TCAGAAAGAT ATTTCGTATAG ATCGTCCTGT TGTACTGGCA ATTAGCGATG
 1301 AGAGTTTTTA TCAAAATGGC TTTTGAATG AGGACCATTG CTATGATGGG
 1351 ATTCTTGAGT TAGATGCTGG GAAAGACATC GTGATTCTCTG CAGATTCTCG
 1401 CAGTATAGAT GCTGTACAAT CTCCGTATGG CTATCAGGGA AAGTGGACGA
 5 1451 TCAATFGGTC TACTGATGAT AAGAAAGCTA CGGTTCTTG GCCGAAGCAG
 1501 AGTTTTTAATC CCACTGCTGA GCAGGAGGCT CCGTTAGTTC CTAATCTTCT
 1551 TTGGGGTTCT TTTATAGATG TTCGTTCCCT CCAGAATTTT ATAGAGCTAG
 1601 GTACTGAAGG TGCTCCTTAC GAAAAGAGAT TTTGGGTTGC AGGCATTTCC
 1651 AATGTTTTGC ATAGGAGCGG TCGTGAAAAT CAAAGGAAAT TCCGTCATGT
 10 1701 GAGTGGAGGT GCTGTAGTAG GTGCTAGCAC GAGGATGCCG GGTGGTGATA
 1751 CCTGTCTCTT GGGTTTTGCT CAGCTCTTTG CGCGTGACAA AGACTACTTT
 1801 ATGAATACCA ATTTTCGCAA GACCTACGCA GGATCTTTAC GTTTGCAGCA
 1851 CGATCTTCC CTATACTCTG TGGTGAGTAT CCTTTTAGGA GAGGGAGGAC
 15 1901 TCCGCGAGAT CCTGTGCCCT TATGTTTCCA AGACTCTGCC GTGCTCTTTC
 1951 TATGGGCAGC TTAGCTACGG CCATACGGAT CATCGCATGA AGACCGAGTC
 2001 TCTACCCCCC CCCCCCCGA CGCTCTCGAC GGATCATACT TCTTGGGGAG
 2051 GATATGTCTG GGCTGGAGAG CTGGGAACTC GAGTTGCTGT TGAAAATACC
 2101 AGCGGCAGAG GATTTTTCCA AGAGTACACT CCATTTGTAA AAGTCCAAGC
 2151 TGTTTACGCT CGCCAAGATA GCTTTGTAGA ACTAGGAGCT ATCAGTCGTG
 20 2201 ATTTTAGTGA TTCGCATCTT TATAACCTTG CGATTCCTCT TGGAATCAAG
 2251 TTAGAGAAAC GGTTCGAGA GCAATATTAT CATGTTGTAG CGATGTATTC
 2301 TCCAGATGTT TGTCGTAGTA ACCCCAAATG TACGACTACC CTACTTTCCA
 2351 ACCAAGGGAG TTGGAAGACC AAAGGTTCCA ACTTAGCAAG ACAGGCTGGT
 2401 ATTGTTTCAGG CCTCAGGTTT TCGATCTTTG GGAGCTGCAG CAGAGCTTTT
 25 2451 CGGGAACCTT GGCTTTGAAT GCGGGGATC TTCTCGTAGC TATAATGTAG
 2501 ATGCGGGTAG CAAAATCAAA TTTTAG

The PSORT algorithm predicts outer membrane (0.92).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 70A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot and for FACS analysis (Figure 70B).

The cp6270 protein was also identified in the 2D-PAGE experiment (Cpn0013).

These experiments show that cp6270 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 71

35 The following *C.pneumoniae* protein (PID 4376402) was expressed <SEQ ID 141; cp6402>:

1 MNVADLLSHL ETLLSSKIFQ DYGPNGLQVG DPQTPVKKIA VAVTADLETI
 51 KQAVAAEAVV LIVHVGIFWK GMPYPITGMI HKRIQLLIEH NIQLIAYHLP
 101 LDAHPTLGNN WRVALDLNWH DLKPFSSSLP YLGVQGSFSP IDIDSFIDLL
 151 SQYYQAPLKG SALGGPSRVS SAALISGGAY RELSSAATSQ VDCFTTGNFD
 40 201 EPAWSTALES NINFLAFGHT ATEKVGPKSL AEHLKSEFPI STTFIDTANP
 251 P*

The cp6402 nucleotide sequence <SEQ ID 142> is:

1 ATGAATGTTG CGGATCTCCT TTCTCATCTT GAGACTCTTC TCTCATCAAA
 51 AATATTTTCAG GATTATGGAC CCAACGGACT TCAAGTTGGA GATCCCCAAA
 45 101 CTCCGGTAAA GAAAATCGCT GTTGCAAGTTA CCGCAGATCT AGAAACCATA
 151 AAACAAGCTG TTGCGGCCGA AGCAAACGTT CTCATGTGAC ACCACGGAAT
 201 TTTTGGGAAA GGTATGCCCT ATCCTATTAC CGGCATGATC CATAAGCGCA
 251 TCCAATTAAT AATAGAACAC AATATCCAAC TCATTGCCTA CCACCTTCCT
 301 TTGGATGCTC ACCCTACCTT AGGAAATAAC TGGAGAGTTG CCCTGGATCT
 50 351 AAATTGGCAT GACTTGAAGC CCTTTGGTTC TTCCCTCCCT TATTTAGGAG
 401 TGCAAGGCTC TTTCTCTCCT ATCGATATAG ATTCTTTTCAT TGACCTGTTA
 451 TCTCAATATT ACCAAGCTCC CCTAAAAGGA TCTGCCCTTG GCGGCCCTC
 501 TAGAGTCTCC TCAGCAGCTC TGATCTCAGG AGGAGCTTAT AGAGAACTCT
 551 CTTCCGGCAGC CACGTCCCAA GTCGATTGCT TCATCACAGG AAATTTTGAT
 55 601 GAACCTGCAT GGTGACAGC TCTAGAAAGC AATATCAACT TCCTAGCATT
 651 TGGACATACA GCCACAGAAA AAGTAGGTCC AAAATCTCTT GCAGAGCATC

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701 TAAAAAGCGA ATTTCTTATT TCCACAACCT TTATAGATAC GGCCAACCCC
751 TTCTAA

The PSORT algorithm predicts cytoplasmic (0.158).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 71A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 71B) and for FACS analysis.

These experiments show that cp6402 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 72

10 The following *C.pneumoniae* protein (PID 4376520) was expressed <SEQ ID 143; cp6520>:

1 MKHYLSFSPS ADFFSKQGA I ETQVLFGERV LVKGSTCYAY SQLFHNELLW
51 KPYPGHSFRS TLVPCTPEFH IHPNVSVVSV DAFLEDFWGP LPFGTLLHVN
101 SQNTVIFPKD ILNHMNTIWG SGTPQCDPRH LRRLNYNFFA ELLIKDADLL
151 LNFPPVWGGG SVHESLEKPG VDCSGFINIL YQAQGYNVPR NAADQYADCH
15 WISSFENLPS GGLIFLYPKE EKRIHVMLK QDSSTLIHAS GGGKKVEYFI
251 LEQDGKFLDS TYLFFRNQGR GRAFFGIPRK RKAFL*

The cp6520 nucleotide sequence <SEQ ID 144> is:

1 ATGAAACACT ACCTATCATT TTCTCCTTCT GCTGATTTTT TCTCTAAACA
51 GGGTGCTATT GAAACTCAAG TCCTTTTTGG AGAGCGCGTC TTAGTCAAAG
201 GGAGCACCTG CTATGCATAT TCCCAATTAT TCCACAATGA GCTGTTATGG
151 AAGCCCTATC CAGGTCATAG CTTTCGTTCT ACCCTAGTCC CCGCACTCC
201 TGAATTTTCA ATCCATCCAA ATGTTCTGTG GGTTCCTGTG GATGCATTTT
251 TAGATCCTTG GGGGATCCCT CTTCCTTTTG GAACTTTACT CCATGTGAAT
301 TCTCAAAATA CCGTTATTTT CCCTAAGGAT ATTCTCAATC ATATGAACAC
251 CATCTGGGGC TCCGGCACAC CTCATGCGA TCCTAGACAT CTACGTCGTC
401 TAAATATATA CTTCTTTGCT GAACTTTTAA TTAAAGACGC AGACCTTTTA
451 CTGAACTTTC CCTATGTATG GGGAGGACGG TCTGTACACG AAAGTCTGGA
501 AAAGCCGGGT GTTGATTTGT CCGGATTTAT CAATATCCTT TACCAGGCAC
551 AGGGATACAA CGTCCCTAGA AACGCTGCAG ATCAATATGC GGATGTCAT
301 TGGATCTCTA GCTTTGAGAA CCTTCCTTCT GGTGGGTAA TATTTCTTTA
651 CCCTAAAGAA GAAAAGCGTA TTTCTCATGT TATGTTGAAA CAGGATAGTT
701 CCACCCTCAT TCATGCTTCT GGTGGAGGGA AAAAAGTGA GTATTTTCAAT
751 TTAGAACAAG ATGGGAAGTT TTTAGATTCT ACTTATCTAT TTTTLAGAAA
801 TAATCAGAGG GGACGGCAT TTTTGGGAT CCCTAGAAAA AGAAAAGCCT
35 851 TTCTGTAA

The PSORT algorithm predicts cytoplasmic (0.265).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 72A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 72B) and for FACS analysis.

40 These experiments show that cp6520 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 73

The following *C.pneumoniae* protein (PID 4376567) was expressed <SEQ ID 145; cp6567>:

1 MTSPPIPFQSS GDASFLEAQP QQLPSTSESQ LVTQLLTMMK HTQALSETVL
45 51 QQQRDLRPTA SIILQVGGAP TGGAGAPFP GPADDDHHPI PPPVVPAQIE
101 TEITTTIRSEL QLMRSTLQOS TKGARTGVLV VTAILMTISL LAIIIIILAV
151 LGFTGVLPQV ALLMQGETNL IWAMVSGSII CFIALIGTLG LILTNNKNTPL

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201 PAS*

The cp6567 nucleotide sequence <SEQ ID 146> is:

1 ATGACCTCAC CGATCCCCTT TCAGTCTAGT GCGATGCCT CTTTCCTTGC
 5 51 CGAGCAGCCA CAGCAACTCC CGTCTACTTC TGAATCTCAG CTAGTAACTC
 101 AATTGCTAAC CATGATGAAG CATACTCAAG CATTATCCGA AACGGTTCTT
 151 CAACAACAAC GCGATCGATT ACCAACCGCA TCTATATATCC TTCAAGTAGG
 201 AGGAGCTCCT ACAGGAGGAG CGGGTGCGCC TTTTCAACCA GGACCGGCAG
 251 ATGATCATCA TCATCCCATA CCGCCGCTG TTGTACCAGC TCAAATAGAA
 301 ACAGAAATCA CCACTATAAG ATCCGAGTGA CAGCTCATGC GATCTACTCT
 10 351 ACAACAAAGC ACAAAGGAG CTCGTACAGG AGTTCTAGTG GTTACTGCAA
 401 TCTTAATGAC GATCTCCTTA TTGGCTATTA TTATCATAAT ACTAGCTGTG
 451 CTTGGATTGA CGGGCGTCTT GCCTCAAGTA GCTTTATTGA TGCAGGGTGA
 501 AACAAATCTG ATTTGGGCTA TGGTGAGCGG TTCTATTATT TGCTTTATTG
 551 CGCTAATTGG AACTCTAGGA TTAATTTTAA CAAATAAGAA CACGCCTCTA
 15 601 CCGGCTTCTT AA

The PSORT algorithm predicts inner membrane (0.694).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 73A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 73B) and for FACS analysis.

- 20 These experiments show that cp6567 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 74The following *C.pneumoniae* protein (PID 4376576) was expressed <SEQ ID 147; cp6576>:

1 MLIMRNKVL QISILALIQT PLTLFSTKEKV KEGHVVDISI TIITEGENAS
 25 51 NKHPLPKLKT RSGALFSQLD FDEDLRLAK BYDSVEPKVE FSEGKTNIAL
 101 HLIAPKPSIRN IHISGNQVVP EHKILKTLQI YRNDLPEREK FLKGLDDLRT
 151 YYLKRGYFAS SVDYSLEHNQ EKGHIDVLIK INEGPCGKIK QLTFSGISRS
 201 EKSDIQEFIQ TKQHSSTTSW FTGAGLYHPD IVEQDSLAIT NYLHNNGYAD
 251 AIVNSHYDLD DKGNIILYMD IDRGSRYYTLG HVHIQGFVFL PKRLIEKQSQ
 30 301 VGPNDLYCPD KIWDGAHKIK QTYAKYGYIN TNVDVLFIPH ATRPIYDVITY
 351 EVSEGSPPYKV GLIKITGNTH TKSDVILHET SLFPDGTFFNR LKLEDTEQRL
 401 RNTGYFQSVS VYTVRSQLDP MGNADQYRDI FVEVKETTTG NLGLFLGFSS
 451 LDNLFGGIEL SESNFDLFGA RNIFSKGFRC LRGGGEHLFL KANFGDKVTD
 501 YTLKWKPHF LNTFWILGIE LDKSINRALS KDYAVQTYGG NVSTTYLINE
 35 551 HLKYGLFYRG SQTSLHEKIK PLLGPNIDSN KGFVSAAGVN LNYDSVDSPR
 601 TPTTGIRGGV TFEVSLGGT YHFTKLSLNS SIYRKLTRKG ILKIKGEAQF
 651 IKPYSNPTAE GVPVSEPFLL GGETTVRGYK SFIIGPKYSA TEPQGLSSSL
 701 LISEEFQYPL IRQPNISAFV FLDSGFVGLQ EYKISLKLDR SSAGFGLRFD
 751 VMNNVFMVLG FGWPFRTPTET LNKEKIDVSQ RFFPALGGMF *

- 40 A predicted signal peptide is highlighted.

The cp6576 nucleotide sequence <SEQ ID 148> is:

1 ATGCTCATCA TCGGAAATAA AGTTATCTTG CAAATATCTA TTCTAGCGTT
 51 AATCCAAACC CCTTTAACTT TATTTTCTAC TGAATAAGTT AAAGAAGGCC
 101 ATGTGGTGGT AGACTCTATC ACAATCATAA CGGAAGGAGA AAATGCTTCA
 45 151 AATAAACATC CCTTACCCAA ATTAAGAGACC AGAAGTGGGG CTCTTTTTC
 201 TCAATTAGAT TTTGATGAAG ACTTGAGAAT TCTAGCTAAA GAATACGACT
 251 CTGTTGAGCC TAAAGTAGAA TTTTCTGAAG GGAAACTAA CATAGCCCTT
 301 CACCTAATAG CTAAACCCTC AATTCGAAAT ATTATATCTT CAGGAAATCA
 351 AGTCGTTCCCT GAACATAAAA TTCTTAAAAA CCTACAAATT TACCGTAATG
 50 401 ATCTCTTTGA ACGAGAAAAA TTTCTTAAGG GTCTTGATGA TCTAAGAACG
 451 TATTATCTCA AGCGAGGATA TTTTCGCATCC AGTGTAGACT ACAGTCTGGA
 501 ACACAATCAA GAAAAAGGTC ACATCGATGT TTTAATTAAA ATCAATGAAG
 551 GTCCTTGGCG GAAAAATTAA CAGCTTACGT TCTCAGGAAT CTCTCGATCA
 601 GAAAAATCAG ATATCCAAGA ATTTATTCAA ACCAAGCAGC ACTCTACAAC

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5 651 TACAAGTTGG TTTACTGGAG CTGGACTCTA TCACCCAGAT ATTGTTGAAC
 701 AAGATAGCTT GGCAATTACG AATTACCTAC ATAATAACGG GTACGCTGAT
 751 GCTATAGTCA ACTCTCACTA TGACCTTGAC GACAAAGGGA ATATTCTTCT
 801 TTACATGGAT ATTGATCGAG GGTCCGCGATA TACCTTAGGA CACGTCCATA
 851 TCCAAGGGTT TGAGGTTTTG CCAAAACGCC TTATAGAAAA GCAATCCCAA
 901 GTCGGCCCCA ATGATCTTTA TTGCCCCGAT AAAATATGGG ATGGGGCTCA
 951 TAAGATCAAA CAAACTTATG CAAAGTATGG CTACATCAAT ACCAATGTAG
 1001 ACGTTCCTTT CATCCCTCAC GCAACCCGCC CTATTTATGA TGTAACCTAT
 1051 GAGGTAAGTG AAGGGTCTCC TTATAAAGTT GGGTTAATTA AAATTACTGG
 1101 GAATACCCAT ACAAATCTG ACGTTATTTT ACACGAAACC AGTCTCTTCC
 1151 CAGGAGATAC ATTCAATCGC TTAAAGCTAG AAGATACTGA GCAACGTTTA
 1201 AGAAATACAG GCTACTTCCA AAGCGTTAGT GTCTATACAG TTCGTTCTCA
 1251 ACTTGATCCT ATGGGCAATG CGGATCAATA CCGAGATATT TTTGTAGAAG
 1301 TCAAAGAAAC AACACAGGA AACTTAGGCT TATTCTTAGG ATTTAGTTCT
 1351 CTTGACAATC TTTTGGAGG AATTGAACTA TCTGAAAGTA ATTTTGATCT
 1401 ATTTGGAGCT AGAAATATAT TTTCTAAAGG TTTTCGTTGT CTAAGAGGCG
 1451 GTGGAGAACA TCTATTCTTA AAAGCCAAC TCGGGGACAA AGTCACAGAC
 1501 TACTACTTTGA AGTGGACCAA ACCTCATTTT CTAACACTC CTTGGATTTT
 1551 AGGAATTGAA TTAGATAAAT CAATTAACAG AGCATTATCT AAAGATTATG
 20 1601 CTGTCCAAAC CTATGGCGGG AACGTCAGCA CAACGTATAT CTTGAACGAA
 1651 CACCTGAAAT ACGGTCTATT TTATCGAGGA AGTCAAACGA GTTTACATGA
 1701 AAAACGTAAG TTCCTCCTAG GGCCAAATAT AGACAGCAAT AAAGGATTTG
 1751 TCTCTGCTGC AGGTGTCAAC TTGAATTACG ATTCTGTAGA TAGTCTTAGA
 25 1801 ACTCCAAC TAAGGATTTC GGGGGGGTG ACTTTTGAGG TTTCTGGTTT
 1851 GGGAGGAACT TATCATTTTA CAAACTCTC TTTAAACAGC TCTATCTATA
 1901 GAAACTTAC GCGTAAAGGT ATTTTGAAAA TCAAAGGGGA AGCTCAATTT
 1951 ATTTAAACCCT ATAGCAATAC TACAGCTGAA GGAGTTCCTG TCAGTGAGCG
 2001 CTTCTTCCTA GGTGGAGAGA CTACAGTTCG GGGATATAAA TCCTTTATTA
 2051 TCGGTCCAAA ATACTCTGCT ACAGAACCTC AGGGAGGACT CTCTTCGCTC
 30 2101 CTTATTTTCAG AAGAGTTTCA ATACCCTCTC ATCAGACAAC CTAATATTAG
 2151 TGCCTTTGTA TTCTTAGACT CAGGTTTGT CCGTTTACAA GAGTATAAGA
 2201 TTTCTGTTAA AGATCTACGT AGTAGTCTG GATTGTGCT CCGCTTCGAT
 2251 GTAAATGAATA ATGTTCTCTGT TATGTTAGGA TTTGGTTGGC CCTTCCGTCC
 2301 AACCAGAGCT TTGAATGGAG AAAAAATTGA TGTATCTCAG CGATTCTTCT
 35 2351 TTGCTTTAGG GGGCATGTTC TAA

The PSORT algorithm predicts outer membrane (0.7658).

The protein was expressed in *E.coli* and purified as GST-fusion (Figure 74A), his-tag and his-tag/GST-fusion products. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 74B) and for FACS analysis (Figure 74C).

40 The cp6576 protein was also identified in the 2D-PAGE experiment (Cpn0300).

These experiments show that cp6576 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 75

The following *C.pneumoniae* protein (PID 4376607) was expressed <SEQ ID 149; cp6607>:

45 1 MNKRQKDKLK ICVIISTLIL VGIFARAPRG DTFKTFKSE EAIIVSNQCN
 51 EDMRKILCDA IEHADREIFL RIYNLSEPKI QQSLTRQAQA KNKVITYYQK
 101 FKIPQILKQA SNVTLVEQPP AGRKLMHQKA LSIDKKDAWL GSANYTNLSL
 151 RLNNLILGM HSELCDLII TNSGDFSIR DQGTGYFVLP QDRKIAIQAV
 201 LEKIQTAKT IQVAMFALH SEIIQALHQA KQGIHVDII IDRSHSKLTF
 50 251 KQLRQLNINK DFVSTINTAPC TLHHKFAVID NKTLLAGSIN WSKGRPSLND
 301 ESLIILENLT KQONQKLRLI WKDLAKHSEH PTVDDDEEKEI IEKSLPVEEQ
 351 EAA*

A predicted signal peptide is highlighted.

The cp6607 nucleotide sequence <SEQ ID 150> is:

-115-

1 ATGAATAAAA GACAAAAAGA TAAATTAAAA ATCTGTGTTA TTATTAGCAC
 51 GTTGATTTTA GTAGGAATTT TTGCAAGAGC TCCTCGTGGT GACACTTTTA
 101 AGACTTTTTT AAAGTCTGAA GAAGCTATCA TCTACTCAA TCAATGCAAT
 5 GAGGACATGC GTAAAATTCT ATGCGATGCT ATAGAACACG CTGATGAAGA
 201 GATCTTCCTA CGTATTTATA ACCTCTCAGA ACCCAAGATC CAACAGAGTT
 251 TAACTCGACA AGCTCAAGCA AAAAACAAAG TTACGATCTA CTATCAAAAA
 301 TTTAAAATTC CCCAAATCTT AAAGCAAGCC AGCAATGTAA CTTTAGTCGA
 351 GCAACCTCCA GCAGGGCGTA AACTGATGCA TCAAAAAGCT CTTTCCATAG
 401 ATAAGAAAGA TGCTTGGCTA GGATCTGCGA ACTACACCAA TCTTTCTCTA
 10 CGTTTAGATA ATAATCTCAT TCTAGGAATG CATAGCTCGG AGCTCTGTGA
 501 TCTCATTATC ACAAATACCT CTGGAGACTT TTCTATAAAG GATCAAACAG
 551 GAAAGTATTT TGTTCCTCCT CAAGATCGTA AAATTGCAAT ACAAGCTGTA
 601 CTCGAAAAAA TCCAGACAGC TCAGAAAACC ATCCAAGTTG CTATGTTTGC
 651 TCTGACCCAC TCGGAGATTA TTCAAGCCTT ACATCAAGCA AAACAACGAG
 15 GAATCCATGT AGATATTATC ATTGATAGAA GTCATAGCAA ACTTACTTTT
 751 AAGCAATTAC GACAAATAAA TATCAATAAA GACTTGTGTT CTATAAATAC
 801 CGCACCTGT ACTCTTCACC ATAAGTTTGC AGTTATAGAT AATAAACTC
 851 TACTTGCAGG ATCTATAAAT TGGTCTAAAG GAAGATTCTC CTTAAATGAT
 901 GAAAGCTTGA TCATACTGGA AAACCTGACC AAACAACAAA ATCAGAAACT
 20 TCGAATGATT TGGAAAGATC TAGCTAAGCA TTCAGAACAT CCTACAGTAG
 1001 ACGATGAAGA AAAAGAAATT ATAGAAAAAA GTCTTCCAGT AGAAGAGCAA
 1051 GAAGCAGCGT GA

The PSORT algorithm predicts periplasmic (0.934).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 75A) and also as a
 25 GST-fusion. The GST-fusion protein was used to immunise mice, whose sera were used in a Western
 blot (Figure 75B) and for FACS analysis.

These experiments show that cp6607 is a surface-exposed and immunoaccessible protein, and that it
 is a useful immunogen. These properties are not evident from the sequence alone.

Example 76

30 The following *C.pneumoniae* protein (PID 4376624) was expressed <SEQ ID 151; cp6624>:

1 MDAKMGYIFK VMRWIFCFVA CGITFGCTNS GFQANSRPC ILSMNRMIHD
 51 CVERVVGNR L ATAVLIKGS L DPHAYEMVKG DKDKLAGSAV IFCNGLGLEH
 101 TSLRLKHLN NPNSVKLGER LIARGAFVPL EEDGICDPHI WMDLSIWKEA
 151 VIEITEVLIE KFPWSAEFK ANSEELVCEM SILDSWAKQC LSTIPENLRY
 35 LVSGHNAFSY FTRRYLATPE EVASGAWRSR CISPEGLSPE AQISVRDIMA
 201 VVDYINEHDV SVVFPEDTLN QDALKKIVSS LKKSHLVRLA QKPLYSDNVD
 251 DNYFSTFKHN VCLITEELGG VALECQR*

The cp6624 nucleotide sequence <SEQ ID 152> is:

1 ATGGATGCGA AAATGGGATA TATATTAAAA GTGATGCGTT GGATTTTCTG
 40 51 TTTCGTGGCA TGTGGTATAA CTTTGGGATG TACCAATTCT GGGTTTCAGA
 101 ATGCAAATTC ACGTCCCTGT ATACTATCCA TGAATCGCAT GATTTCATGAT
 151 TGTGTTGAAA GAGTCGTGGG GAATAGGCTT GCTACCGCTG TTTTGATCAA
 201 AGGATCCTTA GACCCATCATG CGTATGAGAT GGTAAAGGG GATAAGGACA
 251 AGATTGCTGG AAGTGCCGTA ATTTTGTGTA ACGGCCTGGG TCTTGAGCAT
 45 301 ACATTAAAGT TCGGGAAGCA TTTAGAAAAT AATCCCAATA GTGTCAAGTT
 351 AGGGGAGCGG TTGATAGCGC GTGGGGCCTT TGTTCCTCTA GAAGAAGACG
 401 GTATTTCGGA TCCTCATATC TGGATGGATC TTTCTATTTC GAAGGAAGCT
 451 GTCATAGAAA TTACAGAAGT TCTCATTGAA AAGTTCCTG AATGGTCTGC
 501 TGAATTAAAA GCAAATAGTG AGGAACTTGT TTGTGAAATG TCTATTTTAG
 55 551 ATTCTTGGGC GAAACAATGC TTGAGCAGAA TTCCTGAAAA TTACGGTAT
 601 CTGTCTCAG GTCATAATGC GTTCAGTTAC TTTACACGTC GCTATTTAGC
 651 TACTCCTGAA GAAGTGGCTT CCGGAGCATG GAGGTCTCGT TGTATTCTC
 701 CTGAGGCTCT ATCTCCAGAA GCTCAAATCA GTGTTCTGTA TATTATGGCG
 751 GTGTAGATT ATATTAAATG GCATGATGTC AGTGTGGTTT TCCCTGAGGA
 801 TACTCTGAAC CAAGATGCGT TGAATAAAT TGTTCCTCT CTGAAGAAAA
 851 GTCATTTAGT TCGTCTAGCT CAAAACCAT TGTATAGTGA TAATGTGGAC
 901 GACAAATATT TTAGCACCTT TAAACATAAT GTCTGCCTTA TCACAGAAGA

-116-

951 ATTAGGAGGG GTGGCTCTTG AATGTCAAAG ATGA

The PSORT algorithm predicts inner membrane (0.168).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 76A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 76B) and for FACS analysis.

The cp6624 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp6624 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 77

10 The following *C.pneumoniae* protein (PID 4376728) was expressed <SEQ ID 153; cp6728>:

```

1  MKSSVSWLFF SSIPLFSSLS IVAAEVTLDS SNNSYDGSNG TTFTVFSTTD
51  AAAGTTYSLL SDVSPQNA GA LGIPLASGCF LEAGGDLTFQ GNQHALKFAP
101 INAGSSAGTV ASTSAADKNL LFNDFSRLSI ISCPSLLLSP TGQCALKSVG
151 NLSLTGNSQI IFTQNFSSDN GGVINTKNFL LSGTSQFASF SRNQAFSGKQ
15  201 GGVVYATGTI TIENSPGIVS FSQNLAKGSG GALYSTDNCS ITDNFQVIFD
251 GNSAWAAQA QGGAICCTTT DKTVTLTGNK NLSFTNTAL TYGGAISGLK
301 VSIAGGPTL FQSNISGSSA GQGGGAINI ASAGELALSA TSGDITFNNN
351 QVTNGSTSTR NAINIIDTAK VTSIRAATGQ SIYFYDPITN PGTAASTDTL
401 NLNLADANSE IEYGAIVFS GEKLSPTKA LANVTSTIR QPAVLARGDL
20  451 VLRDGVTVTF KDLTQSPGSR ILMGGTTLS AKRANLSLNG LAVNLSSLDG
501 TNKAALKTEA ADKNISLSGT IALIDTEGSE YENHNLKSAS TYPLLELTTA
551 GANTITLGA LSTLTQEP THYGYQGNWQ LSWANATSSK IGSINWTRTG
601 YIPSPERKSN LPLNSLWGNF IDIRSINQLI BTKSSGEPFE RELWLSGIAN
651 FFYRDSMPTR HGFRHISGGY ALGITATTPA EDQLTFAFCQ LFARDRNHIT
25  701 GKNHGDYGA SLYFHTEGL FDIANFLWGR ATRAPVWLSE ISQIPLSPD
751 AKFSYLHTDN HMKTYTDDNS IIKGSWRNDA FCADLGASLP FVISVPYLLK
801 EVEPFVKVQY IYAHQQDFYE RHAEGRAFNR SELINVEIPI GVTFERDSKS
851 EKGTYDLTLM YILDAYRRNP KCQTSLIASD ANWMAYGTNL ARQGFVRAA
901 NHFQVNPME IFQQFAFEVR SSSRNYNTNL GSKFCF*
```

30 The cp6728 nucleotide sequence <SEQ ID 154> is:

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1  ATGAAGTCCT CTGCTCTTGG GTTGTCTTTT TCTTCAATCC CGCTCTTTTC
51  ATCGCTCTCT ATAGTCGCGG CAGAGGTGAC CTTAGATAGC AGCAATAATA
101 GCTATGATGG ATCTAACGGA ACTACCTTCA CGGCTTTTTC CACTACGGAC
151 GCTGCTGCAG GAACTACCTA TTCTTACTT TCCGACGTAT CCTTTCAAAA
35  201 TGCAGGGGCT TTAGGAATTC CCTTAGCCTC AGGATGCTTC CTAGAAGCGG
251 GCGGCGATCT TACTTTCCAA GGAAATCAAC ATGCACTGAA GTTTGCATTT
301 ATCAATGCGG GCTCTAGCGC TGGAACGTGA GCCAGTACCT CAGCAGCAGA
351 TAAGAACTCT CTCTTTAATG ATTTTCTAG ACTCTCTATT ATCTCTTGTC
401 CCTCTCTTCT TCTCTCTCCT ACTGGACAAT GTGCTTTAAA ATCTGTGGGG
40  451 AATCTATCTC TAACTGGCAA TTCCCAAATT ATATTACTC AGAAGTCTC
501 GTCAGATAAC GCGGTGTTA TCAATACGAA AAACCTCTTA TTATCAGGGA
551 CATCTCAGTT TCGGAGCTTT TCGAGAAACC AAGCCTTCAC AGGGAAGCAA
601 GCGGTGTAG TTTACGCTAC AGGAACTATA ACTATCGAGA ACAGCCCTGG
651 GATAGTTTCC TTCTCTCAAA ACCTAGCGAA AGGATCTGGC GGTGCTCTGT
45  701 ACAGCACTGA CAACGTGTCG ATTACAGATA ACTTTCAAGT GATCTTTGAC
751 GGCAATAGTG CTTGGGAAGC CGCTCAAGCT CAGGCGGGGG CTATTGTTTG
801 CACTACGACA GATAAAACAG TGACTCTTAC TGGGAACAAA AACCTCTCTT
851 TCACAAATAA TACAGCATTG ACATATGGCG GAGCCATCTC TGGACTCAAG
901 GTCAGTATTT CCGCTGGAGG TCCTACTCTA TTTCAAAGTA ATATCTCAGG
50  951 AAGTAGCGCC GGTACGGGAG GAGGAGGAGC GATCAATATA GCATCTGCTG
1001 GGGAACTCGC TCTCTCTGCT ACTTCTGGAG ATATTACCTT CAATAACAAC
1051 CAAGTCACCA ACGGAAGCAC AAGTACAAGA AACGCAATAA ATATCATTTGA
1101 TACCGCTAAA GTCACATCGA TACGAGCTGC TACGGGGCAA TCTATCTATT
1151 TCTATGATCC CATCACAAAT CCAGGAACCG CAGCTTCTAC CGACACATTG
55  1201 AACTTAAACT TAGCAGATGC GAACAGTGAG ATCGAGTATG GGGGTGCGAT
1251 TGTCTTTTCT GGAGAAAAGC TTTCCCTTAC AGAAAAGCA ATCGCTGCAA
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1301 ACGTCACCTC TACTATCCGA CAACCTGCAG TATTAGCGCG GGGAGATCTT
1351 GTACTTCGTG ATGGAGTCAC CGTAACTTTC AAGGATCTGA CTCAAAGTCC
1401 AGGATCCCGC ATCTTAATGG ATGGGGGGAC TACACTTAGT GCTAAAGAGG
1451 CAAATCTTTC GCTTAATGGC TTAGCAGTAA ATCTCTCCTC TTTAGATGGA
1501 ACCAACAAGG CAGCTTTAAA AACAGAAGCT GCAGATAAAA ATATCAGCCT
1551 ATCGGGAACG ATTGCGCTTA TTGACACGGA AGGGTCATTC TATGAGAATC
1601 ATAACTTAAA AAGTGCTAGT ACCTATCCTC TTCTTGAAC TACCACCGCA
1651 GGAGCCAACG GAACGATTAC TCTGGGAGCT CTTTCTACCC TGACTCTTCA
1701 AGAACCTGAA ACCCACTACG GGTATCAAGG AAACCTGGCAG TTGTCTTGGG
1751 CAAATGCAAC ATCCTCAAAA ATAGGAAGCA TCAACTGGAC CCGTACAGGA
1801 TACATTCCCTA GTCCTGAGAG AAAAAGTAAT CTCCCTCTAA ATAGCTTATG
1851 GGGAAACTTT ATAGATATAC GCTCGATCAA TCAGCTTATA GAAACCAAGT
1901 CCAGTGGGGA GCCTTTTGAG CGTGAGCTAT GGCTTTCAGG AATTGCGAAT
1951 TTTCTTCTATA GAGATTCTAT GCCCACCCTG CATGGTTTCC GCCATATCAG
2001 CGGGGGTTAT GCACTAGGGA TCACAGCAAC AACTCCTGCC GAGGATCAGC
2051 TTACTTTTGC CTTCTGCCAG CTCCTTGCTA GAGATCGCAA TCATATTACA
2101 GGTAAGAACC ACGGAGATAC TTACGGTGCC TCTTTGTATT TCCACCATAC
2151 AGAAGGGGCT TTCGACATCG CCAATTTCCT CTGGGGAAAA GCAACCCGAG
2201 CTCCCTGGGT GCTCTCTGAG ATCTCCAGA TCATTCTTTT ATCGTTCGAT
2251 GCTAAATTCA GTTATCTCCA TACAGACAAC CACATGAAGA CATATATAC
2301 CGATAACTCT ATCATCAAGG GTTCTTGGAG AAACGATGCC TTCTGTGCAG
2351 ATCTTGGAGC TAGCTGCCT TTTGTTATTT CCGTTCCGTA TCTTCTGAAA
2401 GAAGTCGAAC CTTTGTGCAA AGTACAGTAT ATCTATGCGC ATCAGCAAGA
2451 CTCTACGAG CGTCATGCTG AAGGACGCGC TTTCAATAAA AGCGAGCTTA
2501 TCAACGTAGA GATTCCCTATA GGCGTCACCT TCGAAAGAGA CTCAAATCA
2551 GAAAAGGGAA CTTACGATCT TACTCTTATG TATATACTCG ATGCTTACCG
2601 ACGCAATCCT AAATGTCAA CTTCCCTAAT AGCTAGCGAT GCTAACTGGA
2651 TGGCCTATGG TACCAACCTC GCACGACAAG GTTTTCTGT TCGTGTGCG
2701 AACCATTTC AAGTGAACCC CCACATGGAA ATCTCGGTC AATTGCTTT
2751 TGAAGTACGA AGTTCTTCAC GAAATTATAA TACAAACCTA GGCTCTAAGT
2801 TTTGTTTCTA G

```

The PSORT algorithm predicts inner membrane (0.187).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 77A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 77B) and for FACS analysis.

The cp6728 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp6728 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 78

40 The following *C.pneumoniae* protein (PID 4376847) was expressed <SEQ ID 155; cp6847>:

45
50

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1 MFVMKKLVRL CVVLLSLLPN VLFSSDLLRE EGIKKMMDKL IEYHVDAQEV
51 STDILSRSL SYIQSFDPHK SYLSNQEAV FLQSPETKKR LLKNYKAGNF
101 AIYRNINQLI HESILRARQW RNEWVKNPKE LVLEASSYQI SKQPMQWSKS
151 LDEVKQRQRA LLLSYLSLHL AGASSRYEG KEEQLAALCL RQIENHENVY
201 LGINDHGVAM DRDEEAYQPH IRVVKALAHS LDAHTAYFSK DEALAMRIQL
251 EKGCMGIGVV LKEDIDGVVV REIIPGGPAA KSGDLQLGDI IYRVDGKDIE
301 HLSFRGVLDL LRGGHGSTVV LDIHRGESDH TIALRREKIL LEDRRVDVSY
351 BPGDGVIGK VTLHSFYEGE NQVSSEQDLR RAIQGLKEKN LLGLVLDIRE
401 NTGGFLSQAI KVSGLFMTNG VVVVSRYADG TMKCYRTVSP KKFYDGPLAI
451 LVSKSSASAA EIVAQTLQDY GVALVVGDEQ TYGKGTIQHQ TITGDASQDD
501 CFKVTVGKYY SPGKSTQLQ GVKSDILIPS LYAEDRLGER FLEHPLPADC
551 CDNVLDHPLT DLDTQTRPWF QKYLPNLQK QETLWREMLP QLTKNSEQRL
601 SENSNFQAFI SQIKSSEKTD LSYGSNDLQL EBSINILKDM ILLQQCRK*

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A predicted signal peptide is highlighted.

55 The cp6847 nucleotide sequence <SEQ ID 156> is:

-118-

1 ATGTTTCGTAA TGAAAAAACT TGTCCGTCTA TGCCTAGTTC TTCTTTCTTT
 51 ACTTCCGAAT GTATTATTTT CTTCCGATCT TTTACGAGAA GAGGGCATCA
 101 AAAAGATGAT GGACAAGCTG ATCGAGTATC ATGTCGATGC TCAAGAGGTT
 151 TCTACGGATA TACTCTCGCG TTCTTTATCT AGTTACATT C AATCTTTTGA
 5 201 TCCTCATAAA TCTTATCTTT CAAACCAAGA GGTTCGAGTT TTCTACAGT
 251 CTCCGGAAC AAAGAAACGT CTCTTAAAGA ATTATAAGGC AGGCAACTTT
 301 GCTATTTATC GCAACATCAA TCAATTAAT CATGAGAGTA TTCTTCGTGC
 351 CAGGCAGTGG AGAAACGAAT GGGTTAAGAA TCCAAAAGAG CTTGTATTGG
 401 AGGCATCCTC ATATCAGATA TCGAAGCAAC CTATGCAATG GAGCAAATCT
 10 451 TTAGACGAAG TGAAGCAGAG ACAACGCGCT CTACTCCTTT CCTATCTTTC
 501 TTTACATCTT GCTGGAGCTT CTTCTCTCTG TTATGAGGGT AAAGAAGAGC
 551 AGCTTGCTGC TCTGTGTCTA CGTCAAATCG AGAACCATGA GAATGTATAT
 601 TTAGTATCA ACGATCATGG TGTGCTATG GATCGGGATG AAGAAGCCTA
 651 CCAATTCCAT ATCCGTGTTG TTAAAGCTTT AGCTCATAGC TTAGATGCAC
 15 701 ATACGGCGTA TTTCACTAAG GACGAAGCGT TGGCGATGCG AATCCAACCTA
 751 GAAAAAGGCA TGTGTGGAAT TGGTGTGTGT CTGAAGGAAG ATATTGATGG
 801 AGTTGTGTGT AGAGAAATCA TTCTGGGGG ACCTGCGGCT AAATCTGGGG
 851 ATCTTCAGCT TGGAGATATC ATCTATCGGG TGGATGGCAA GGATATCGAG
 901 CATCTTTCTT TCCGCGGTGT TTTAGATTGT TTACGTGGAG GTCATGGCTC
 20 951 TACTGTAGTC TTAGATATCC ATCGTGGGGA GAGCGATCAT ACGATCGCCT
 1001 TGAGAAGGGA GAAAACTCTT TTAGAAGACC GTCGTGTGGA TGTTCCTTAT
 1051 GAGCCTTATG GAGATGGTGT GATTGGGAAA GTTACGTTAC ATTCTTTTTA
 1101 TGAAGGAGAA AATCAGGTTT CTAGTGAACA AGATCTACGT CGAGCGATTC
 1151 AGGGATTAAA GGAGAAGAAC CTTCTTGGAT TAGTTTTAGA TATCCGAGAA
 25 1201 AATACGGGTG GATTTTATAT TCAAGCGATC AAAGTTTCTG GTTATTTAT
 1251 GACCAATGGC GTTGTGGTTG TATCTCGCTA TGCTGATGGT ACCATGAAGT
 1301 GCTACCGCAC AGTATCTCCT AAAAAATTCT ATGATGGTCC TTGGCTATT
 1351 TTAGTATCTA AAAGTTCCGC ATCAGCAGCG GAGATTGTAG CACAACTCT
 1401 CCAAGATTAT GGAGTTGCTT TAGTTGTTGG AGATGAGCAG ACCTATGGGA
 30 1451 AGGGAACGAT TCAGCATCAA ACAATTACTG GAGATGCCTC TCAGGACGAT
 1501 TGTTTTAAAG TTAATGTAGG GAAATATTAT TCCCTTCTG GGAAATCGAC
 1551 TCAACTTCAG GGAGTAAAT CCGATATTTT AATTCCTTCT CTCTATGCTG
 1601 AAGATCGTCT AGGAGAGCGT TTTCTAGAGC ATCCCTTACC TGCAGATTGC
 1651 TGTGATAATG TACTTCACGA TCCTCTCACG GACTTGGATA CTCAAACACG
 35 1701 TCCTTGGTTT CAAAAATACT ATCTTCCTAA TCTACAAAAG CAAGAGACTC
 1751 TTTGGAGAGA GATGCTACCT CAGCTTACGA AAAACAGTGA GCAAAGGCTT
 1801 TCTGAGAATT CGAATTTFCA GGCATTTTGT TCGCAGATAA AATCATCTGA
 1851 AAAAACGGAC CTATCTATG GTTCCAATGA TTTACAATTG GAAGAGTCGA
 1901 TAAACATTTT GAAGGACATG ATTTTATTAC AACAGTGTAG AAAATAA

40 The PSORT algorithm predicts periplasmic (0.932).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 78A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 78B) and for FACS analysis.

45 These experiments show that cp6847 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 79

The following *C.pneumoniae* protein (PID 4376969) was expressed <SEQ ID 157; cp6969>:

1 MRLFSLGTYI LFFSLALSSC CGYSILNSPY HLSSLGKSL L QERIFIAPIK
 51 EDPHGQLCSA LTYELSKRSF AISGRSSCAG YTLKVELLNG IDKNIGFTYA
 101 PNKLGDKTHR HFIVSNEGR L SLSAKVQLIN NDTQEV LIDQ CVARESVD F D
 151 FEPLDGTANA HEFALGQFEM HSEAIKSARR ILSIRLAETI AQQVYVDLF*

A predicted signal peptide is highlighted.

The cp6969 nucleotide sequence <SEQ ID 158> is:

55 1 ATGAGATTGT TTCTTTTAGG CACGATTTAT CTTTTTTTTT CTCTAGCACT
 51 TTCGTCATGC TGTGGTFACT CTATTTTAAA CAGCCCGTAT CACTTATCGT
 101 CTTTAGGTAA GTCTTTATTA CAGGAAAGAA TTTTCATTGC TCCCATAAAA

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151  GAAGATCCTC  ATGGTCAGCT  CTGCTCAGCT  CTAACCTATG  AGCTTAGTAA
201  GCGTTCCTTT  GCTATCTCTG  GAAGGAGTTC  TTGCGCAGGC  TATACTCTTA
251  AAGTAGAGCT  TCTGAATGGT  ATTGACAAGA  ATATAGGTTT  TACGTATGCC
301  CCAAATAAAC  TCGGAGATAA  GACTCACAGG  CATTTTATAG  TCTCTAATGA
5    351  AGGCAGACTA  TCACTATCTG  CAAAAGTACA  GCTTATCAAT  AATGACACTC
401  AAGAAGTCCT  TATAGACCAA  TGTGTTGCTC  GAGAGTCTGT  AGACTTTGAC
451  TTTGAGCCTG  ACTTAGGAAC  AGCAAACGCT  CATGAATTG  CTTTAGGCCA
501  ATTTGAAATG  CATAGTGAAG  CCATAAAAG  TGCTCGCCGT  ATACTATCTA
551  TACGCCTAGC  CGAGACGATT  GCTCAACAGG  TATACTATGA  CCTTTTTTGA

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10 The PSORT algorithm predicts inner membrane (0.126).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 79A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 79B) and for FACS analysis.

15 These experiments show that cp6969 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 80

The following *C.pneumoniae* protein (PID 4377109) was expressed <SEQ ID 159; cp7109>:

```

1  MKKTCCQNYR  SIGVVFSSVL  FVLTTQTLFA  GHFIDIGTSG  LYSWARGVSG
51  DGRVVVGYEG  GNAFKYVDGE  KFLLEGLVPR  SEALVFKASY  DGSVIIGISD
20  101  QDPSCRAVKW  VNGALVDLGI  FSEGMQSFAB  GVSSDGKTIV  GCLYSDDTET
151  NFAVKWDETF  MVVLEPNLPD  RHSCAWDASE  DGSVIVGDAM  GSEELAKAVY
201  WKDGEQHLLS  NIPGAKRSSA  HAVSKDGSFI  VGEFISEENE  VHAFVYHNGV
251  IKDIGTLGGD  YSVATGVSRD  GKVIVGHSTR  TDGEYRAFKY  VDGRMIDLGT
301  LGGSASFAPG  VSDDGKTIIV  KFE TELGECH  APTIYLLDD*

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25 A predicted signal peptide is highlighted.

The cp7109 nucleotide sequence <SEQ ID 160> is:

```

1  ATGAAAAAGA  CATGTTGCCA  AAATTACAGA  TCGATAGGCG  TTGTGTTCTC
51  TGTGTTACTT  TTCGTTCTTA  CAACACAGAC  GCTGTTTGCA  GGACATTTTA
101  TTGATATTGG  AACTTCTGGA  TTATATTCTT  GGGCTCGAGG  TGTATCTGGA
30  151  GATGCGCCCG  TTGTCGTAGG  TTATGAAGGT  GGCAATGCAT  TTAAATATGT
201  TGATGGTGAG  AAATTCTGTG  TAGAAGGTTT  GGTCCCGAGA  TCCGAGGCCT
251  TGGTATTTAA  AGCTTCTTAT  GATGGCTCTG  TAAATTATAGG  AATCTCGGAT
301  CAAGATCCGT  CTTGCGCGCG  TGTGAAGTGG  GTAAACGGTG  CACTTGTGTA
351  TCTTTGGAATA  TTTTCTGAGG  GAATGCAATC  TTTTGCAAG  GGTGTTTCCA
35  401  GTGATGGA  GACGATTGTA  GGGTGCCTAT  ATAGTGATGA  TACAGAGACA
451  AACTTTGCTG  TGAAGTGGGA  TGAAACAGGA  ATGGTTGTTT  TCCCTAACTT
501  ACCAGAAGAT  CGACATCTTT  GCGCTTGCGA  TGCCCTCTGAA  GATGGCTCTG
551  TGATTGTAGG  GGACGCCATG  GGTAGCGAGG  AAATTGCCAA  GGCAGTGTAC
601  TGGAAAGGACG  GTGAACAACA  TCTGCTTTCT  AATATCCCAG  GAGCTAAAAG
40  651  ATCGTCAGCA  CATGCAGTTT  CTAAGATGG  ATCTTTTATC  GTAGGCGAGT
701  TCATCAGTGA  AGAAAATGAA  GTTCATGCCT  TTGTTTATCA  CAACGGTGTT
751  ATCAAAGATA  TCGGGACTTT  AGGAGGAGAT  TACTCTGTAG  CAACTGGAGT
801  TTCTAGGGAT  GGTAAAGTCA  TCGTGGGTCA  TTCTACAAGA  ACAGATGGTG
851  AATACCGTGC  ATTTAAATAT  GTGGATGGAA  GAATGATAGA  TTTGGGGACT
45  901  TTAGGAGGTT  CAGCATCTTT  TGCTTTTGGT  GTTCTTGACG  ATGGCAAAC
951  AATCGTAGGA  AAATTGAAA  CAGAGCTAGG  AGAATGTCAT  GCCTTTATCT
1001  ACCTTGATGA  TTAG

```

The PSORT algorithm predicts outer membrane (0.887).

50 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 80A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 80B) and for FACS analysis.

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These experiments show that cp7109 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 81

The following *C.pneumoniae* protein (PID 4377110) was expressed <SEQ ID 161; cp7110>:

```

5      1  MAAIKQILRS  MLSQSSLWMV  LFSLYLSGY  CYVITDKPED  DFHSSSAVKW
      51  DHWGKTTLRS  LSNKKASAKA  VSGTGATTVG  FIKDTWSRTY  AVRWNWYGTK
     101  ELPTSSWVKK  SKATGISSDG  SIIAGIVENE  LSQSFVAVTWK  NNEMYLLPST
     151  WAVQSKAYGI  SSDGSVIVGS  AKDAWSRTFA  VKWTGHEAQV  LPVGWAVKSV
     201  ANSVSANGSI  IVGSVDASG  ILYAVKWEGN  TITHLGLTGG  YSAIAKAVSN
    10  251  NGKVIIVGRSE  TYYGEVHAF  HKNGVMSDLG  TLGGSYSAAK  GVSATGKIVIV
     301  GMSTTANGKL  HAFKYVGGRM  IDLGEYSWKE  ACANAVSIDG  EIIIVGVQSE*
```

A predicted signal peptide is highlighted.

The cp7110 nucleotide sequence <SEQ ID 162> is:

```

15      1  ATGGCAGCTA  TAAAACAAAT  TTTACGTTCT  ATGCTATCTC  AGAGTAGCTT
      51  ATGGATGGTC  CTATTTTCAT  TATATTTCTCT  ATCTGGTTAT  TGCTATGTAA
     101  TTACAGACAA  ACCAGAAGAT  GACTTCCATT  CTTTATCCGC  AGTAAAATGG
     151  GATCATTGGG  GAAAGACAAC  TCTCTCAAGA  TTATCAAATA  AAAAAGCCTC
     201  TGCAAAAGCT  GTTTCAGGAA  CTGGTGCTAC  AACTGTCGGC  TTTATAAAAG
     251  ACACCTTGGT  TCGAACATAC  GCAGTAAGAT  GGAATTATTG  GGGGACCAAA
     301  GAACTCCCTA  CCAGCTCATG  GGTAAGAAAA  TCAAAAGCAA  CAGGAATCTC
     351  CTCTGATGGG  TCTATAATCG  CGGGGATTGT  CGAGAATGAG  CTTTCTCAAA
     401  GTTTCGCAGT  CACATGGAAA  AACAAAGAAA  TGTATTGCT  CCCTTCCACA
     451  TGGGCAGTGC  AATCTAAAGC  GTATGGAATT  TCTTCTGATG  GCTCTGTTAT
     501  TGTAGGGAGT  GCTAAGGATG  CTTGGTCGCG  AACTTTCGCT  GTGAAGTGGA
    25  551  CGGGACACGA  GGCTCAGGTG  TTACCAGTAG  GCTGGGCTGT  CAAATCTGTA
     601  GCGAATTCTG  TATCTGCCAA  TGGATCTATA  ATTGTAGGGT  CTGTACAAGA
     651  CGCCTCTGGA  ATTCTTTATG  CTGTAAAGTG  GGAAGGGAAC  ACTATTACAC
     701  ATCTAGGAAC  TTTAGGAGGC  TATTCTGCCA  TTGCAAAAGC  TGTATCCAAT
     751  AATGGCAAGG  TCATTGTAGG  GAGATCCGAA  ACATATTATG  GAGAGGTCCA
    30  801  TGCATTCTGT  CATAAGAATG  GCGTCATGTC  AGACCTCGGC  ACCCTCGGAG
     851  GATCTTATTC  TGCAGCTAAG  GGAGTCTCTG  CAACTGGAAA  AGTTATTGTC
     901  GGTAGTGCCA  CAACAGCAAA  TGGGAAATTG  CATGCCTTTA  AATATGTCGG
     951  TGGAAGAATG  ATCGACTTAG  GAGAGTATAG  CTGGAAAGAA  GCCTGTGCAA
    1001  ACGCTGTTTC  TATTGATGGA  GAAATTATTG  TTGGAGTCCA  ATCAGAATAA
```

35 The PSORT algorithm predicts outer membrane (0.827).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 81A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 81B) and for FACS analysis.

40 These experiments show that cp7110 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Figure 191 shows a schematic representation of the structural relationships between of cp7105, cp7106, cp7107, cp7108, cp7109 and cp7110, each of which is identified herein. These six proteins may be grouped in a new family of related outer membrane-associated proteins. These proteins have a repeat structure in common (*cf.* the pmp family).

45 Example 82

The following *C.pneumoniae* protein (PID 4377127) was expressed <SEQ ID 163; cp7127>:

```

1  MVFFRNSLLH  LVALSGMLCC  SSGVALTIAE  KMASLEHSGR  GADDYEGMAS
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51 FNANMREYSL QLSKLYBEAR KLRASGTEDE ALWKDLIRRI GEVRGYLREI
 101 EELWAAEIRE KGGNLEDYAL WNHPEPTTIYN LVTDYGTEDS IYLIPOEIGA
 151 IKIATLSKFV VPKESEFEDCL TQILSRLGIG VRQVNSWIKE LYMMRKEGCS
 201 VAGVFSSRKD LEALPETAYI GFVLNSNVDA HTNQHVLLKF INPETHVDV
 5 251 IAGRUVIFGS AGEVGELLKI YNFVQSESR QEYRVIPLTK IDPGEMISIL
 301 NAAPREDLTK DVSEESLGLR VVPLQYQGRS LFLSGTAALV QQALTLIREL
 351 EEGIENPTDK TVFWYNVKHS DPQELAALLS QVHDVPSGEN KASVGAADGC
 401 GSQLNASIQI DTTVSSSAKD GSVKYGNFIA DSKTGTLMV VEKEVLPRIQ
 451 MLLKKLDVPR KMVRIEVLFF ERKLAHEQKS GLNLLRLGEE VCKKGCSPSV
 10 501 SWAGGTGILE FLFKGSTGSS IVPGYDLAYQ FLMAQEDVRI NASPSVVTMN
 551 QTPARIAVVD EMSIAVSSDK DKAQYNRAQY GIMIKMLPVI NVGEEDGKSY
 601 ITLETDTITFD TTGNHDDRP DVTRRNITNK VRIADGETVI IGGLRCQMS
 651 DSHDGIPFLG DIPGIGKLF MSSTSDSLTE MFVFITPKIL ENPVEQQRK
 701 EEALLSSRPG EREYYQALA ASEAAARAAH KKLEMPASG VLSQVERQE
 15 751 YDGC*

A predicted signal peptide is highlighted.

The cp7127 nucleotide sequence <SEQ ID 164> is:

1 ATGGTPTTTT TCCGTAATTC TTTACTGCAT TTAGTGTCCC TATCCGGAAT
 51 GCTCTGTTGT TCTTCTGGAG TGGCTTTAAC GATAGCCGAG AAGATGGCTT
 20 101 CTTTAGAGCA CTCGGGGAGA GGAGCAGACG ATTATGAGGG GATGGCTTCG
 151 TTTAATGCCA ATATGAGGGA GTATAGCCTT CAGCTGAGCA AGTTGTATGA
 201 GGAAGCACGA AAGCTACGCG CTTCTGGAAC TGAGGATGAA GCTCTGTGGA
 251 AGGACTTAAT TCGACGGATT GGTGAGGTGC GAGGCTATCT TCGAGAGATC
 301 GAGGAGCTTT GGGCTGCAGA AATTCTGTGAG AAAGGGGGCA ATCTCGAGGA
 25 351 CTACGCCCTC TGGAAATCACC CAGAGACTAC GATTTACAAT CTTGTTACCG
 401 ATTACGGAAC CGAAGACTCT ATTTATTTGA TTCTCAAGA AATCGGAGCG
 451 ATTAATAATCG CAACCTTATC GAAATTTGTA GTTCTAAAG AGTCTTTTCA
 501 AGACTGTCTC ACTCAGATCC TATCTCGCTT AGGTATTGGC GTGCGTCAGG
 551 TCAATCTCTG GATTAAGGAA CTTTATATGA TCGTAAGGA GGGCTGCAGT
 30 601 GTTGCTGGAG TTTTTCCTC CAGAAAAGAT TTAGAGGCGC TCCAGAAAC
 651 AGCCTATATT GGTTTTGTAT TGAATTCGAA CGTAGATGCG CATACCAATC
 701 AACATGTCTT AAAAAAGTTC ATTAACCTTG AAACAACGCA TGTAGATGTG
 751 ATTGCAGGAC GTGTGTGGAT TTTTGGTTCT GCGGGGGAAG TCGGCGAGCT
 801 TCTGAAGATT TATAATTTTG TGCAGTCGGA GAGCATACGT CAAGAGTATC
 35 851 GGGTGATTCC CTTAACTAAG ATCGATCCAG GGGAGATGAT TTCCATCTC
 901 AACGCAGCAT TTCGTGAGGA TCTGACTAAA GATGTTAGTG AAGAATCTTT
 951 AGGCCTTCGT GTAGTTCCCT TACAGTATCA AGGCCTTCG TTGTTTAA
 1001 GTGGAACCGC GCGCTTAGTG CAGCAAGCGC TGAATCTCAT TCGAGAGCTT
 1051 GAAGAAGGGA TTGAGAACCC TACGGATAAA ACAGTATTTT GGTATAACGT
 40 1101 CAAGCACTCC GATCCCCAAG AGTTGGCGGC ATTGCTTTCC CAAGTCCATG
 1151 ATGTCTTCTC TGGCGAGAAT AAGCGAGGTG TCGGAGCTGC AGATGGATGT
 1201 GGGTCGCAAT TAAATGCCTC GATCCAAATT GATACTACAG TAAGTTCTTC
 1251 TGCGAAAGAT GGCTCAGTGA AGTACGGAAA CTTTATCGCG GATTCTAAGA
 1301 CAGGAATCTT GATTATGGTG GTTGAGAAAG AAGTTCTTCC ACGTATTCAG
 45 1351 ATGCTACTTA AGAACTAGA TGTCCCTAAA AAGATGGTCC GTATCGAGGT
 1401 GCTGTTATTT GAAAGAAAAT TGGCACATGA GCAGAAATCT GGGTTAAATC
 1451 TTCTACGTCT TGGTGAGGAA GTTTGTAAAA AAGGGTGCAG TCCTTCTGTG
 1501 TCTTGGGCCG GGGGTACTGG CATACTAGAA TTTTATTTA AAGGAAGTAC
 1551 GGGATCTTCG ATAGTTCCCT GTTATGATCT CGCCTATCAA TTTTAAATGG
 50 1601 CTCAAGAGGA CGTTCGGATT AATGCGAGTC CTTCTGTAGT TACTATGAAC
 1651 CAAACCCAG CACGGATTGC TGTGTTGAT GAAATGTCAA TAGCGGTGTC
 1701 TTCAGATAAA GATAAGCGC AATACAATCG TCGCGAGTAC GGTATCATGA
 1751 TAAAAATGCT CCCCATAATT AATGTGGGAG AGGAAGACGG AAAAAGTTAC
 1801 ATTACTTTAG AGACAGACAT CACCTTTGAT ACTACGGGA AAAATCATGA
 55 1851 TGATCGTCTT GATGTTACAA GCGGTAATAT TACTAATAAG GTGCGATTG
 1901 CTGACGAGGA GACTGTGATT ATTGGAGGTT TGGCTTGCAA ACAGATGTCA
 1951 GATTCTCATG ATGGCATTCC TTTCTTTGGA GACATTCCTG GTATAGGGAA
 2001 GTTATTTGGA ATGAGTTCCA CATCAGACAG TCTCACGGAG ATGTTTGTAT
 2051 TTATCACTCC GAAGATCCTA GAAAACTCTG TAGAGCAACA AGAACGTAAA
 60 2101 GAAGAAGCTT TACTCTCTTC GCGCCCTGGA GAGAGAGAAG AATACTATCA
 2151 GGCTTTAGCA GCTAGTGAGG CTGCAGCACG AGCAGCTCAT AAAAAATTAG
 2201 AGATGTTCCC GGCATCAGGA GTATCTTTAT CTCAGGTAGA GAGGCAAGAA
 2251 TACGATGGCT GCTAG

The PSORT algorithm predicts periplasmic (0.920).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 82A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 82B) and for FACS analysis.

These experiments show that cp7127 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 83

The following *C.pneumoniae* protein (PID 4377133) was expressed <SEQ ID 165; cp7133>:

```

1  MQPFIFTLLC L1TSLSVSLVAF DAANARKRCA CAQTIERGEN FFSIKRSACA
51  EIEYQEKSRH ASAIERISKD KGKVTPKQIA KVATKKKQRY RLLQVPFSRP
101 PNNSRYNLYA LLSEPPPECYS DTASWYAIFI RLLRRAYVDI GNVPPGSEYA
151 IANALISNKQ EILERGAQLG PDVIETLTLP EEQAEIFYKM LKGSNSQSL
201 LNFLHYREKS LGHCKLNLIF MDPLLEAVL DHPDAYRETS LLRDGIWEAV
251 KRQEHAIQEH GQAAALELFK TRTDFRLELR DKMQLLSRY DLLPLLNKRM
301 FDYTLGSAGD YLFLVDPDTK AISRCRCPSK SIKL

```

A predicted signal peptide is highlighted.

The cp7133 nucleotide sequence <SEQ ID 166> is:

```

1  ATGCAACCTT TTATCTTTAC TTTACTGTGC TTGACATCTT TGGTTTCTTT
51  AGTCGCCTTT GATGCTGCGA ATGCTCGTAA ACGTTGTGCC TGTGCTCAAA
101 CTATAGAACG TGGAGAGAAC TTCTTTTCCA TAAAACGCTC TGCTTGTGCT
151 GAAATCGAAT ATCAAGAAAA ATCTCGCCAC GCCTCAGCAA TTGAAAGAAT
201 CTCAAAGAT AAAGGCAAG TCACTCCAAA GCAGATTGCG AAAGTAGCTA
251 CTAAGAAAAA GCAAAGATAC CGTTTATTGC AGGTTCTTTT TTCAAGGCCT
301 CCGAATAACT CAAGGTATAA CCTCTATGCT TTGCTTAGTG AACCTCCCGA
351 ATGCTATAGC GATACAGCAT CATGGTATGC TATTTTATT TCGTTACTTC
401 GACGTGCTTA TGTAGACACG GGAAATGTAC CTCCTGGATC TGAGTATGCC
451 ATCGCTAATG CTTTGATAAG TAACAAACAA GAGATTTTAG AGAGGGGAGC
501 GCAGCTTGGG CCCGATGTTA TTGAAACTCT AACATTGCC T GAGGAACAAG
551 CCGAGATTTT TTATAAAATG CTCAAAGGGT CGTCAAAC TCAGTCGCTA
601 CTGAATTTTC TGCATTATGA AGAGAAAAGC TTAGGCCACT GTAAGCTAAA
651 TCTGATCTTC ATGGATCCCC TACTGTTAGA AGCTGTTCTA GATCATCCCG
701 ATGCTTATAG GGAACGTCG CTCTGCGCG ATGGCATTG GGAAGCGGTG
751 AAGCGTCAAG AACATGCCAT CCAAGAACAT GGCCAGGCAG CTGCTTTGGA
801 GCTTTTTAAA ACACGCACCG ACTTCGCCT GGAGCTGCGA GATAAGATGC
851 AGTTACTTCT AAGTCGATAC GATTTGCTCC CCTTATTAAA TAAAAAATG
901 TTCGACTACA CCTTAGGAAG TGCCGAGAT TACTTATTTT TGGTAGACCC
951 AGATACTAAG GCAATTTCTC GATGTCGCTG CCCTTCAAAG AGTATTAAAT
1001 TATAA

```

The PSORT algorithm predicts outer membrane (0.92).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 83A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 83B) and for FACS analysis.

These experiments show that cp7133 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 84

The following *C.pneumoniae* protein (PID 4377222) was expressed <SEQ ID 167; cp7222>:

```

1  MNRRDMVITA VVVNAILLVA LFVTSKRIGV KDYDEGFRNF ASSKVTOAVV
51  SEEKVIEKPV VAEVPSRPIA KETLAAQFIE SKPVIVTTPP VPVSETPEV

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101 PTVAVPPQPV RETVKEEQAP YATVVVKGD FLERIANH TTVAKLMQIN
 151 DLTTTQLKIG QVIKVFPSQD VSNEKTPQTQ TANPENYYIV QEGDSPWTIA
 201 LRNHIRLDDL LKMNDLDEYK ARRLKPGDQL RIR*

A predicted signal peptide is highlighted.

5 The cp7222 nucleotide sequence <SEQ ID 168> is:

1 ATGAATCGTA GAGACATGGT AATAACAGCT GTCGTAGTGA ATGCTATATT
 51 GCTTGTGGCT CTTTTCGTCA CATCAAAGCG TATTGGCGTC AAGGACTATG
 101 ACGAGGGATT CCGTAATTTT GCTTCTAGCA AGGTTACACA AGCAGTAGTT
 151 TCAGAAAGAA AAGTCATAGA AAAGCCTGTA GTCGCAGAAG TGCCTAGCCG
 10 201 TCCTATCGCT AAAGAGACTC TAGCTGCACA GTTTATTGAA AGTAAGCCGG
 251 TTATTGTAAAC CACACCACCC GTGCCTGTTG TTAGCGAAAC CCCAGAAGTG
 301 CCTACTGTGG CAGTTCGCC TCAGCCTGTT CGTGAGACAG TAAAAGAGGA
 351 ACAAGCTCCT TATGCTACTG TTGTAGTGAA AAAAGGAGAT TTTCTCGAAC
 401 GCATTCGCGAG AGCAAATCAT ACTACCGTTG CAAAATTGAT GCAGATCAAT
 15 451 GATCTTACCA CCACCCAAC TAAAAATGGT CAGGTCATCA AAGTCCCTAC
 501 GTCTCAAGAT GTCAGCAACG AAAAACTCC TCAAACACAG ACCGCAAAAC
 551 CTGAAATTA TTATATCGTC CAAGAAGGGG ATAGCCCGTG GACAATAGCA
 601 TTGCGTAACC ATATTCGATT GGATGATTG CTAATAATGA ATGATCTCGA
 651 TGAATATAAA GCCCGCGGCC TTAAGCCTGG AGATCAGTTG CGCATACGTT
 20 701 GA

The PSORT algorithm predicts periplasmic (0.935).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 84A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 84B) and for FACS analysis.

25 These experiments show that cp7222 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 85

The following *C.pneumoniae* protein (PID 4377225) was expressed <SEQ ID 169; cp7225>:

30 1 MKGTPQYHFI GIGGIGMSAL AHILLDRGYE VSGSDLYESY TIESLKAKGA
 51 RCFSGHDSH VPHDAVVVYS SSIAPDNVEY LTAIQRSSRL LHRÆLLSQL
 101 MEGYESILVS GSHGTGTSS LIRALFQEAQ KDPSYAIGGL AANCLNGYSG
 151 SSKIFVAREAD BSDGSLKHYT PRAVVITNID NEHLNNYAGN LDNLVQVIQD
 201 PSRKVTDLNLK VFYNGDCPIL KGNVQGISYG YSPECQLHIV SYNQKAWQSH
 35 251 PSFTFLGOEY QDIELNLPQG HNAANAAAAC GVALTFGIDI NIIRKALKKF
 301 SGVHRRLEK NISESFLFLE DYAHHPVEVA HTLRSVRDAV GLRRVIAIFQ
 351 PHRFSRLLEC LQTFPKAFQE ADEVILTDVY SAGESPRESI ILSDLAEQIR
 401 KSSYVHCCYV PHGDIVDYLK NYIRIHDVCV SLGAGNIYTI GEALKDFNPK
 451 KLSIGLVCGG KSCEHDISLL SAQHVSKYIS PEFYDVSYFI INRQGLWRTG
 501 KDFPHLIEET QGDSPLSSEI ASALAKVDCL FVVLHGPFGE DGTIQGFFEI
 40 551 LGKPYAGPSL SLAATAMDKL LTKRIASAVG VPVVPYQPLN LCFWKRNPKL
 601 CIONLIETFS FPMIVKTAHL GSSIGIFLVR DKEELQEKIS EAFLYDIDVF
 651 VEESRLGSRE IEVSCIGHSS SWYCMAGFNE RCGASGFIDY KEKYGFDGID
 701 CAKISFDLQL SQESLDCVRE LAERVYRAMQ GKGSARIDFF LDEEGNYWLS
 751 EVNPIPGMTA ASPFLQAFVH AGWTQEIVD HFIIDALHKF DRQQTIEQAF
 45 801 TKEQDLVKR*

The cp7225 nucleotide sequence <SEQ ID 170> is:

1 ATGAAGGGAA CTCCTCAGTA TCATTTTATC GGTATCGGTG GTATAGGAAT
 51 GAGCGCTTTA GCTCATATTT TGCTTGATCG TGGCTATGAG GTCTCTGGAA
 101 GCGACTTATA TGAAAGCTAT ACGATCGAAA GCCTGAAAGC TAAAGGTGCG
 50 151 AGGTGTTTCT CAGGCCATGA TTCCTCCCAT GTTCCTCATG ATGCCGTCGT
 201 TGTTTATAGC TCAAGTATAG CCCCTGATAA TGTAGAGTAT CTTACCGCTA
 251 TTCAAAGATC ATCAGTCTTT CTTTCATAGAG CAGAGCTCTT GAGTCAGCTT
 301 ATGGAGGGTT ATGAAAGCAT TCTGGTTTCA GGAAGCCATG GGAAGACAGG
 351 GACCTCATCT CTAATTCGAG CGATTTTTCCA GGAAGCTCAG AAAGATCCCT

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401 CCTATGCTAT TGGAGGACTC GCTGCAAACT GCCTGAATGG GTATTCTGGA
 451 TCATCGAAAA TCTTCGTTCG CGAAGCCGAT GAAAGTGATG GGTCCTTTAAA
 501 GCACCTACACT CCCCGTGCAG TAGTCATTAC AAATATAGAT AATGAACATT
 551 TGAATAATTA CGCTGGGAAT CTTGATAACC TGGTTCAGGT AATCCAGGAC
 5 601 TTCTCTAGAA AAGTAACAGA TCTCAATAAG GTATTCTATA ACGGGGATTG
 651 TCCTATTTTG AAAGGAAATG TCCAAGGGAT TTCTTATGGA TATTCACCAG
 701 AATGTCAATT GCATATCGTT TCCTATAATC AAAAGGCATG GCAATCTCAC
 751 TTTTCCTTTA CCTTTTATAG CCAGGAGTAT CAAGACATTG AGCTCAATCT
 801 CCCTGGACAA CATAACGCTG CAAATGCAGC AGCAGCCTGT GGAGTTGCTC
 10 851 TTACCTTTGG CATAGACATA AACATCATTG GAAAAGCTCT CAAAAAATTC
 901 TCGGGAGTTC ATCGACGTCT AGAAAGAAAA AATATATCCG AAAGCTTTCT
 951 TTTCTTAGAA GATTATGCTC ATCATCCTGT AGAGGTTCGA CATACCCTGC
 1001 GCTCTGTGCG TGATGCTGTG GGTTCGCGAA GAGTCATCGC AATTTTCAA
 1051 CCACATCGAT TCTCTCGTTT AGAAGAGTGC TTACAAACCT TCCCCAAAGC
 15 1101 TTTCCAAGAA GCTGATGAAG TCATACTTAC AGATGTCTAT AGTGCCTGGAG
 1151 AAAGTCCTAG AGAGTCTATC ATTCTTTCCG ACCTTGCGGA ACAGATTTCGT
 1201 AAGTCTTCTT ATGTCCATTG TTGTTATGTT CCCCATGGAG ACATCGTAGA
 1251 TTATCTACGA AACTACATTG GCATTATGA TGCTGTGTT TCTCTAGGAG
 1301 CTGGAAATAT CTATACTATT GGAGAGGCTT TAAAAGACTT TAACCCTAAA
 20 1351 AAATATCCA TAGGACTCGT CTGTGGAGGG AAATCTGCG AACACGATAT
 1401 TTCTCTACTT TCTGCTCAAC ATGCTCTTAA ATATATTCTT CCTGAATTCT
 1451 ATGATGTGAG TTACTTCATC ATAAATCGTC AGGGCTTATG GAGAACAGGA
 1501 AAGGATTTTC CTCATCTTAT TGAAGAGACT CAAGGGGATT CGCCACTTTC
 1551 TTCTGAAATC GCTTCAGCTT TAGCAAAAGT CGACTGTTTG TTTCCCGTGC
 25 1601 TCCATGGCCC ATTTGGAGAG GATGGTACGA TCCAGGGATT TTTTGAAATC
 1651 TTAGGAAAAC CTTATGCCGG ACCCTCACTA TCTTTAGCAG CAACTGCAAT
 1701 GGATAAGCTG TTAACAAAAC GAATTGCATC AGCAGTGGGT GTTCCTGTAG
 1751 TCCCTTACCA ACCTTTAAAT CTCTGTTTCT GGAACGCAA TCCAGAACTA
 1801 TGTATTGAGA ATCTTATAGA GACATTTTCT TTCCCTATGA TTGTAAAAAC
 30 1851 TGCACATTTC GGATCTAGTA TTGGGATATT TTTAGTCCGT GATAAAGAGG
 1901 AATTACAAGA AAAGATCTCA GAAGCATTTT TATATGACAC GGATGTGTTT
 1951 GTGGAGGAAA GTCGCTTAGG GTCTCGTGAA ATCGAAGTGT CCTGTATCGG
 2001 CCATTTCTCT AGCTGGTATT GTATGGCAGG GCCTAATGAA CGCTGTGGTG
 2051 CTAGTGGGTT TATTGATTAT CAAGAGAAAT ATGGATTGTA TGGCATAGAT
 35 2101 TGCGCAAAGA TCTCTTTTGA TTACAGCTC TCACAAGAAT CTTTAGATTG
 2151 TGTTAGAGAA CTTGCAGAGC GTGTCTACCG AGCAATGCAA GGAAGGTT
 2201 CAGCTCGAAT AGATTTTTC TTGGATGAAG AGGGGAATTA TTGGTTGTCA
 2251 GAGGTCAATC CTATTCAGG AATGACAGCA GCTAGCCCAT TTTTACAAGC
 2301 TTTTGTTCAC GCAGGATGGA CGCAAGAACA AATTGTAGAT CACTTTATTA
 40 2351 TAGATGCTCT ACATAAGTTT GATAAGCAGC AGACTATCGA ACAGGCATTG
 2401 ACTAAGAAC AAGATTAGT TAAAAGATAA

The PSORT algorithm predicts inner membrane (0.16).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 85A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 85B) and for FACS analysis.

These experiments show that cp7225 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 86

The following *C.pneumoniae* protein (PID 4377248) was expressed <SEQ ID 171; cp7248>:

50 1 MKFWLQGCAP VGCLLLTLPC CAARRRASGE NLQOTRPIAA ANLQWESYAE
 51 ALEHSKQDHR PICLFFTGSD WCMWCIKMD QILQSSEFKH FAGVHLHMVE
 101 VDFPQKNHQP EEQRQKNQEL KAQYKVTGFP ELVFIDAEGK QLARMGFEPG
 151 GGAAYVSKVK SALKLR*

A predicted signal peptide is highlighted.

55 The cp7248 nucleotide sequence <SEQ ID 172> is:

1 ATGAAATTTT GGTGCAAGG ATGTGCTTTT GTCGGTTGTC TGCTATTGAC

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51   TTTACCTTGT TGTGCTGCAC GAAGACGTGC TTCTGGAGAA AATTTGCAAC
101  AAACCTGCTC TATAGCAGCT GCAAATCTAC AATGGGAGAG CTATGCAGAA
151  GCTCTTGAAC ATTCTTAAACA AGATCACAAA CCTATTGTGC TTTTCTTTAC
201  AGGATCAGAC TGGTGTATGT GGTGCATAAA AATGCAAGAC CAGATTTTGC
5   251  AAAGCTCTGA GTTTAAGCAT TTTGCGGGTG TGCATCTGCA TATGGTTGAA
301  GTTGATTTCC CCCAAAAGAA TCATCAACCT GAAGAGCAGC GCCAAAAAAA
351  TCAAGAACTG AAAGCTCAAT ATAAAGTTAC AGGATTCCCC GAACTGGTCT
401  TCATAGATGC AGAAGGAAAA CAGCTTGCTC GCATGGGATT TGAGCCTGGT
10  451  GGTGGAGCTG CTTACGTAAG CAAGGTGAAG TCTGCTCTTA AACTACGTTA
501  A

```

The PSORT algorithm predicts periplasmic (0.932).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 86A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 86B) and for FACS analysis.

15 The cp7248 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp7248 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 87

The following *C.pneumoniae* protein (PID 4377249) was expressed <SEQ ID 173; cp7249>:

```

20   1  MIPSPFPINF RDDTILETDP KPSLIMFSSK KTEIASERRK AHPTLFKVLG
51   TIWNIVKFII SIILFLPLAL LWVLKKTQCF FILPSSIISQ SMSKTAVAIR
101  RMTFLSHIKQ LLSLKEISAA DRVVIQYDDL VVDSLAIKIP HALPHRWILY
151  SQGNSGLMEN LFDGRDSSLH QLAKATGSNL LVFNYPGIMS SKGEAKRENL
201  VKSYQACVRY LRDEETGPKA NQIIAFGYSL GTSVQAAALD REVTDGSDGT
25  251  SWIVVKDRGP RSLADVANI CKPIASAIK LVGWNIDSVK PSERLRCPFI
301  FIYNSNHDQE LISDGLFERE NCVATPFLEL PEVKTSGETKI PIPERDLLHL
351  NPLSPNVVDR LAAVISNYLD SENRKSQQPD *

```

The cp7249 nucleotide sequence <SEQ ID 174> is:

```

30   1  ATGATCCCAT CCCCTACCCC AATAAACTTT CGTGATGATA CGATTCTAGA
51   GACGGATCCA AAGCCGTCTT TAATCATGTT CTCTTCAAAA AAAACAGAGA
101  TAGCTTCTGA AAGACGGAAG GCCCATCCCA CCTTATTTAA AGTTCTAGGA
151  ACGATTTGGA ATATTTGTGA GTTTATTATC TCAATCATTC TGTTCCTTCC
201  CTTAGCGTTA TTGTGGGTAC TCAAGAAAAC CTGTCAGTTT TTCATTCTCC
25  251  CATCTTCTAT CATATCTCAG AGCATGTCAA AAACAGCTGT GGCAATTCCG
301  CGAATGACCT TTCTGTCCCA TATTAAACAA CTCCTAAGCC TTAAGGAAAT
35  351  CTCAGCTGCC GATCGTGTGG TTATACAATA TGACGATTIG GTGGTTGATA
401  GCTTAGCTAT AAAGATACCT CATGCTCTTC CCCACAGGTG GATTCCTTAT
451  TCTCAAGGAA ACTCTGATT GATGGAAAAC CTGTTTCGATC GGGCGGATTC
501  CTCCTACAC CAGCTAGCCA AAGCAACCGG CTCGAATCTT CTTGTGTTCA
55  551  ACTATCCTGG AATTATGTCC AGCAAAGGAG AAGCGAAACG AGAAATCTG
601  GTTAAATCGT ATCAGGCATG CGTACGCTAC CTACGAGATG AAGAGACAGG
651  TCCTAAAGCC AATCAAATCA TAGCTTTCGG ATACTCTTTG GGAAGTAGTG
701  TCCAAGCTGC TGCTCTAGAT CGTGAGGTCA CTGATGGCAG TGATGGAAC
45  751  TCATGGATTG TTGTAAAAGA TCGGGGCCCT CGCTCTCTAG CAGATGTGCG
801  GAATCAAATT TGTAAGCCCA TAGCTTCCGC GATTATAAAA CTCGTGGGTT
851  GGAACATAGA CTCTGTGAAA CCTAGCGAAA GATTGCGTTG TCCCGAAATT
901  TTCATTACA ACTCTAATCA TGATCAAGAA CTCATTAGCG ACGGCCTCTT
951  CGAAAGAGAA AATTGCGTAG CAACACCTTT TCTAGAGCTT CCTGAAGTAA
1001 A AACCTCGGG GACTAAAATT CCTATACCCG AAAGGATCT TCTCCATCTA
50  1051 AATCCTCTCA GTCCAAATGT AGTAGACAGA TTAGCAGCAG TGATCTCTAA
1101 TTATTTAGAT TCTGAAAACA GAAAGTCTCA GCAACCTGAT TAA

```

The PSORT algorithm predicts inner membrane (0.571).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 87A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 87B) and for FACS analysis.

These experiments show that cp7249 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 88

The following *C.pneumoniae* protein (PID 4377261) was expressed <SEQ ID 175; cp7261>:

```

1  MLPISILLFY VILGCL SAYI ADKKKRVVIG WFFAGAFFGF IGLVVL LLLP
51  SRRNALEKPO NDPFDNSDLF DDLKKS LAGN DEIPSSGDLQ EIVIDTEKWF
101 YLNKDRENVG PISFEELVVL LKGKTYPEEI WVKRGMKDW QRVKDVPSLQ
151 QALKRASK*
```

The cp7261 nucleotide sequence <SEQ ID 176> is:

```

1  ATGCTCCCTA TTTTCGATTTT ATTATTTTAT GTGATTCTAG GTTGTCTATC
51  TGCCTACATA GCAGATAAGA AAAAACGAAA TGTATTGGC TGGTTT TTTG
101 CAGGAGCATT TTTTGGATTT ATTGGTCTAG TTGTCCTTCT TCTTCTTCCT
151 TCTCGTCGAA ACGCTTTAGA AAAGCCACAA AACGATCCTT TTGATAACTC
201 CGATCTTTTT GATGATTGTA AAAAAAGTTT AGCAGGTAAT GACGAGATAC
251 CCTCATCGGG AGATCTTCAA GAAATCGTTA TCGATACAGA GAAGTGGTTT
301 TATTTAAATA AAGATAGAGA AAACGTAGGT CCGATATCTT TTGAGGAGTT
20  351 - GGTCGTACTT TTAAAGGGAA AAACGTATCC AGAAGAAATT TGGGTATGGA
401 AAAAGGGAAT GAAAGATTGG CAACGAGTGA AGGATGTTC ATCACTACAA
451 CAGGCTTTGA AAGAAGCATC AAAATAA
```

The PSORT algorithm predicts inner membrane (0.848).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 88A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 88B) and for FACS analysis.

These experiments show that cp7261 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 89

The following *C.pneumoniae* protein (PID 4377305) was expressed <SEQ ID 177; cp7305>:

```

1  MEVYSFHPAV RTSFQHRVMA ALDAWFFLGG HRLKVVSLDS CNSGWAYQEL
51  VSISTTEKVL KLLSYLLVPI VIIALLIRCL LHSNFRIDVE KERWLKIREL
101 GIDIESCKLP SSVVNQVSSF IWFEKDKSKR PRIDVDYHTL HSKDWVVPPI
151 VFQKIPKTSR FSYWFSQKET RKRDYVRNML DHVIGYLTSE GGEWLQYISK
35  201 TSYQSATSLD PERVLQYCLT DNQELQGEVQ RLLNERSATK SSGDKKVVLS
251 HVSDIICQCW WPKFLEVIQS PAFIEELVEE VSGKLNLDPL CLEKANTLDQ
301 ELRNSLLRAV VHHGSEGVDI KKVAGGLIY TEAIQLQIPF SRS*
```

The cp7305 nucleotide sequence <SEQ ID 178> is:

```

1  ATGGAAGTTT ATAGTTTTC A CCTGCGGTA AGGACTTCGT TTCAGCACCG
40  51  TGTAATGGCA GCACTAGATG CTTGGTTT T TCTAGGAGGG CACCGTTTAA
101 AAGTAGTTTC TCTAGATAGT TGTAAGTCAG GTTGGGCGTA TCAAGAACTT
151 GTGTCTATTT CAACGACAGA AAAAGTCTTG AAAGTACTCT CTTACCTACT
201 CGTACCGATT GTCATAATAG CTCTGTTAAT TCGTTGTCTT TTACATAGCA
251 ATTTTAGGAT AGACGTAGAG AAGGAACGTT GGTAAAAAAT AAGGGAGTTA
45  301 GGAATTGATA TAGAAAGCTG CAAACTCCCC AGTTCCTTATG TAAACCAGGT
351 TTCTCTGTTT ATTTGTTTG AAAAAGATAA ATCCAAACGG CCACGTATTG
401 ATGTAGATTA TCATACGCTA CATAGCAAAG ACTGGGTAGT TTTCCCTATC
```

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451 GTTTTTCAGA AAATTCACAA GACCTCGCGT TTCAGTTATT GGTTCACACA
 501 AAAAGAAACA AGGAAGAGGG ATTATGTGAG AAATATGCTG GACCACGTCA
 551 TTGCTTATCT AACGTCAGAA GGTGGGGAGT GGTTCAGTA TATATCGAAA
 5 601 ACCCTCTTATC AAAGCGCTAC TTCTTGGAT CCTGAAAGAG TTCTTCAATA
 651 TTGCTTAACT GATAACCAGG AGCTCCAGGG AGAAGTGCAA CGTTTGCTTA
 701 ATGAGGAGAG TCGGACCAAA AGCTCTGGGG ATAAGGAAGT TTTGTTAAGT
 751 CATGTATCTG ACATTATTTG CCAGTGTGG TGGCCAAAGT TTCTTGAAGT
 801 TATACAATCT CCGGCCTTTA TTGAAGAATT AGTAGAAGAA GTGAGTGGTA
 851 AACTTAATTT AGATTTTTTA TGCCTAGAAA AGGCTAATAC ATTAGATCAG
 10 901 GAGTTGAGAA ACAGTCTTCT AAGAGCAGTC GTACACCACG GTTCTGAAGG
 951 AGTTGATATT AAGAAAGTTG GTGCCGGCCT CATTATTTAT ACGGAAGCTA
 1001 TTCAATTACA GATTCCCTTC TCAAGGAGTT AA

The PSORT algorithm predicts inner membrane (0.508).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 89A) and also as a
 15 double GST/his fusion. The recombinant proteins were used to immunise mice, whose sera were
 used in a Western blot (Figure 89B) and for FACS analysis.

These experiments show that cp7305 is a surface-exposed and immunoaccessible protein, and that it
 is a useful immunogen. These properties are not evident from the sequence alone.

Example 90

20 The following *C.pneumoniae* protein (PID 4377347) was expressed <SEQ ID 179; cp7347>:

1 MKKGLGAIIV FGLLFTSSVA GFSKDLTKDN AYQDLNVIEH LISLKYAPLP
 51 WKELLFGWDL SQQTQQARLQ LVLEEKPTTN YCQKVLNRYV RSLNDYHAGI
 101 TFYRTESAYI PYVLKLSERG HVFVVDVQTS QGDIYLGDEI LEVDGMGIRE
 151 AIESLRFGRG SATDYSAAVR SLTSRSAAFG DAVPSGIAML KLRRPSGLIR
 201 STPVRWRYTP RHIGDFSLVA PLIPEHKPQL PTQSCVLFPS GVNSQSSSSS
 25 251 LFSSYMPYYP WEELRVQNKQ RFDSNHHIGS RNFGLPTFGP ILWEQDKGPY
 301 RSYIFKAKDS QGNPHRIGFL RISSYVWTDL EGLEBDHKDS PWELFGEIID
 351 HLEKETDALI IDQTHNPGRS VFYLYSLLSM LTDHPLDTFK HRMIFQDEV
 401 SSALHWQDLL EDVFTDEQAV AVLGETMEGY CMDMHAVASL QNFSQSVLSS
 30 451 WVGSDINLSK FMPLLGFAQV RPHPKHQYTK PLFMLIDEDD FSCGDLAPAI
 501 LKDNGRATLI GKPTAGAGGF VFQVTFPNRS GIKGLSLTGS LAVRKDGEFI
 551 ENLGVAPHID LGFTSRDLQT SRFTDYVEAV KTIIVLTSLSE NAKKSEBQTS
 601 PQETPEVIRV SYPTTTSAS*

A predicted signal peptide is highlighted.

35 The cp7347 nucleotide sequence <SEQ ID 180> is:

1 ATGAAAAAAG GGAAATTAGG AGCCATAGTT TTTGGCCTTC TATTTACAAG
 51 TAGTGTGCT GGTTTTCTA AGGATTGAC TAAAGACAAC GCTTATCAAG
 101 ATTTAAATGT CATAGAGCAT TTAATATCGT TAAAATATGC TCCTTTACCA
 151 TGGAAAGAAC TATTATTTGG TTGGGATTTA TCTCAGCAAA CACAGCAAGC
 40 201 TCGCTTGCAA CTGGTCTTAG AAGAAAAACC AACAACCAAC TACTGCCAGA
 251 AGGTACTCTC TAACTACGTG AGATCATTA ACGATTATCA TGCAGGGATT
 301 ACGTTTATC GTACTGAAAG TCGGTATATC CCTTACGTAT TGAAGTTAAG
 351 TGAAGATGGT CATGTCTTTG TAGTCGACGT ACAGACTAGC CAAGGGGATA
 401 TTTACTTAGG GGATGAAATC CTTGAAGTAG ATGGAATGGG GATTCTGTAG
 45 451 GCTATCGAAA GCCTTCGCTT TGGACGAGGG AGTGCCACAG ACTATTCTGC
 501 TGCAGTTCGT TCCTTGACAT CGCGTTCGCG CGCTTTTGGA GATGCGGTTC
 551 CTTCAGGAAT TGCCATGTTG AAACCTCGCC GACCCAGTGG TTTGATCCGT
 601 TCGACACCGG TCCGTTGGCG TTATACTCCA GAGCATATCG GAGATTTTTC
 651 TTTAGTTGCT CCTTTGATTC CTGAACATAA ACCTCAATTA CCTACACAAA
 50 701 GTTGTGTGCT ATTCCGTTCC GGGGTAAATT CACAGTCTTC TAGTAGCTCT
 751 TTATTAGATT CCTACATGGT GCCTTATTTT TGGGAAGAAT TCGGGGTICA
 801 AAATAAGCAG CGTTTGTGACA GTAATCACCA TATAGGGAGC CGTAATGGAT
 851 TTTTACCTAC GTTTGGTCCT ATTCTTTGGG AACAAGACAA GGGGCCCTAT
 901 CGTTCCCTATA TCTTTAAAGC AAAAGATTCT CAGGGCAATC CCCATCGCAT
 55 951 AGGATTTTTA AGAATTCTCT CTTATGTTTG GACTGATTTA GAAGGACTTG
 1001 AAGAGGATCA TAAGGATAGT CCTTGGGAGC TCTTTGGAGA GATCATCGAT

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1051  CATTTGGAAG  AAGAGACTGA  TGCTTTGATT  ATTGATCAGA  CCCATAATCC
1101  TGGAGGCAGT  GTTTTCTATC  TCTATTCGTT  ACTATCTATG  TTAACAGATC
1151  ATCCTTTAGA  TACTCCTAAA  CATAGAATGA  TTTTCACTCA  GGATGAAGTC
1201  AGCTCGGCTT  TGCAGTGGCA  AGATCTACTA  GAAGATGTCT  TCACAGATGA
1251  GCAGGCAGTT  GCCGTGCTAG  GGGAAACTAT  GGAAGGATAT  TGCATGGATA
1301  TGCATGCTGT  AGCCTCTCTT  CAAAACCTCT  CTCAGAGTGT  CCTTTCTTCC
1351  TGGGTTTCAG  GTGATATTAA  CCTTTCAAAA  CCTATGCCCT  TGCTAGGATT
1401  TGCACAGGTT  CGACCTCATC  CTAAACATCA  ATATACTAAA  CCTTTGTTTA
1451  TGTTGATAGA  CGAGGATGAC  TTCTCTGTGT  GAGATTTAGC  GCCTGCAATT
1501  TTGAAGGATA  ATGGCCGCGC  TACTCTCAT  GGAAAGCCAA  CAGCAGGAGC
1551  TGGAGGTTT  GTATTCCAAG  TCACCTTCCC  TAACCGTTCT  GGAATTAAAG
1601  GTCTTTCCTT  AACAGGATCT  TTAGCTGTTA  GGAAAGATGG  TGAGTTTATT
1651  GAAACCTTAG  GAGTGGCTCC  TCATATTGAT  TTAGGATTTA  CCTCCAGGGA
1701  TTTTGCAAACT  TCCAGGTTTA  CTGATTACGT  TGAGGCAGTG  AAAACTATAG
1751  TTTTAACTTC  TTTGTCTGAG  AACGCTAAGA  AGAGTGAAGA  GCAGACTTCT
1801  CCGCAAGAGA  CGCCTGAAGT  TATTCGAGTC  TCTTATCCCA  CAACGACTTC
1851  TGCTTCGTAA

```

The PSORT algorithm predicts periplasmic space (0.2497).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 90A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 90B) and for FACS analysis.

These experiments show that cp7347 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 91

The following *C. pneumoniae* protein (PID 4377353) was expressed <SEQ ID 181; cp7353>:

```

1  MNMPVPSAVP  SANITLKEDS  STVSTASGIL  RTATGEVLVS  CTALEGSST
51  DALISLALGQ  IILATQOELL  LQSTNVHQLL  FLPPEVVELE  IQVVDLLVQL
101  EHAETITSEP  QETQTSRSE  QTLPOQSSSK  QSALSPRSLK  PEISDSKQQQ
151  ALQTPKDSAV  RKHSEAPSPE  TQARASLSQA  SSSSQSLPP  QESAPERTLL
201  EQQKASSFSP  LSQPSAEKQK  EALTTSKSHE  LYKERDQDRQ  QREQHDRKHD
251  QEEDAESKKK  KKKRGLGVEA  VAEPEGENLD  IALIFSDQM  RPPAETSCK
301  ETTFFKKLPS  PMSVPSRFIP  SKNPLSVGSS  IHGPIQTPKV  ENVFLRFMKL
351  MARILGQARA  EANELYMRVK  QRTDDVDTLT  VLISKINNER  KDIDWSENER
401  MKALLNRAKE  IGVTTDKKEY  TWTREEKRLL  KENVQMRKEN  MEKITQMERT
451  DMQRHLQEIS  QCHQARSNVL  KLLKELMDTF  IYNLRP*

```

The cp7353 nucleotide sequence <SEQ ID 182> is:

```

1  ATGAATATGC  CTGTTCCCTC  TGCAGTCCCC  TCTGCAAATA  TAACTCTAAA
51  AGAAGACAGC  TCAACAGTTT  CCACAGCCTC  TGGAAATATTA  AAGACTGCAA
101  CAGGTGAAGT  CTTAGTCTCT  TGTACAGCGC  TAGAAGGAAG  CTCTTCTACA
151  GATGCTTTAA  TTAGCTTAGC  TTTAGGACAA  ATCATTCTTG  CGACCCAACA
201  AGAAGTGCTC  TTACAAAGCA  CAAATGTTCA  TCAACTCCTC  TTCTTCCCTC
251  CTGAAGTTGT  AGAATTAGAA  ATCCAAGTTG  TTGACTTGCT  AGTGCAATTG
301  GAACATGCAG  AGACAATCAC  AAGTGAACCA  CAAGAAACAC  AAACGCAAAG
351  TAGGAGTGAG  CAGACCCTCC  CTCACAAAG  CAGCAGTAAA  CAATCTGCTC
401  TCTCCCCACG  CTCCTTAAAA  CCTGAAATTT  CTGATTCTAA  ACAACAGCAA
451  GCTCTTCAAA  CACCAAAAAG  CTCTGCTGTA  AGAAAACACA  GCGAAGCACC
501  GTCACCTGAG  ACACAAGCTC  GCGCTTCTTT  ATCTCAGGCA  AGCTCAAGTT
551  CTCAGAGATC  CTTACCTCCG  CAAGAAAGTG  CGCCAGAAAG  AACACTATTA
601  GAACAACAAA  AAGCAAGCTC  CTTCTCTCCT  CTATCCAGT  TCTCTGCAGA
651  GAAACAAAAA  GAGGCCCTGA  CGACCTCAAA  ATCTCATGAA  CTCTATAAAG
701  AACGCGATCA  AGATCGCCAA  CAAAGAGAGC  AGCAGCAGAG  AAAGCAGCAT
751  CAGGAAGAAG  ACGCTGAATC  TAAAAAGAAA  AAGAAGAAAC  GTGGTCTCGG
801  TGTAGAGGCA  GTCGCTGAGG  AACC CGGAGA  AAATCTAGAT  ATTGCCGCTT
851  TAATCTTCTC  AGATCAAATG  CGACCTCCTG  CTGAAGAAAC  TTCTAAAAAA
901  GAAACGACAT  TCAAAAAGAA  GCTACCTTCT  CCAATGTCTG  TGTTTAGCAG
951  ATTATCCCT  AGTAAGAATC  CGTTATCTGT  AGGCTCTTCA  ATACACGGGC
1001  CTATACAAAC  TCCAAAAGTA  GAAAATGTGT  TCTTAAGGTT  CATGAAGCTC

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```

1051 ATGGCAAGAA TCTTAGGCCA AGCCGAAGCC GAAGCTAATG AACTCTACAT
1101 GCGAGTCAAA CAACGTACCG ATGATGTAGA CACACTCACA GTCCTTATCT
1151 CTAAGATCAA TAATGAAAAG AAAGACATTG ATTGGAGTGA AAATGAAGAG
1201 ATGAAAGCTC TTTTAAATCG AGCTAAAGAG ATTGGAGTCA CTATAGACAA
1251 AGAAAAATAT ACTTGGACAG AAGAGGAAAA AAGACTTCTA AAAGAGAATG
1301 TCCAAATGCG CAAAGAGAAAT ATGGAGAAAA TCACTCAAAT GGAAAGGACG
1351 GACATGCAAA GGCACCTCCA AGAGATTCTT CAATGTCATC AAGCGCGCTC
1401 TAATGTATTG AAGTTATTGA AAGAACTTAT GGACACCTTC ATTTACAACC
1451 TACGCCCCTA A

```

10 The PSORT algorithm predicts cytoplasm (0.1308).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 91A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 91B) and for FACS analysis.

15 These experiments show that cp7353 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 92

The following *C.pneumoniae* protein (PID 4377408) was expressed <SEQ ID 183; cp7408>:

```

20 1 MLKIQKKRMC VSVVITVGAI VGFNSADAA PKKKKIPIQI LYSFTKVSSY
51 LKNEASTIF CVDVDRGLLQ HRYLGSPGWQ ETRRRQLFKS LENQSYGNER
101 LGERTLAIDI FRNKECLESE IPEQMEAILA NSSALVLGIS SFGITGIPAT
151 LHSLLRQNLQ FQKRSIASES FLLKIDSAPS DASVFKGVL FRGETAIVDA
201 LSQLPQQLDL SPKKIIFLGE DPEVVQAVGS ACIGWGMNPL GLVYYPQES
251 LFSYVHPYST ATELQEAQGL QVISDEVAQL TLNALPKMN*

```

The cp7408 nucleotide sequence <SEQ ID 184> is:

```

25 1 ATGTTGAAAA TCCAGAAAAA AAGAATGTGT GTCAGCGTAG TCATCACGGT
51 AGGCCCCATA GTGGGGTTTT TCAATTCTGC AGACGCGACA CCAAAGAAAA
101 AGAAGATCCC TATACAGATT CTCTACTCCT TTAATAAAGT CTCCTCCTAT
151 TTAAAAACG AAGACGCAAG TACTATATTT TGCGTCGATG TGGATCGTGG
201 ACTTCTCCAG CATCGGTATT TAGGTAGTCC AGGATGGCAG GAAACCAGAC
30 251 GTCGCGAGTT ATTTAAATCC TTAGAAAATC AATCATACGG CAACGAACGT
301 TTAGGAGAAG AAACCTCTGC TATTGATATT TTCAGGAACA AAGAGTGCTT
351 GGAGAGCGAG ATCCAGAGC AGATGGAAGC TATCCTTGCA AATTCTCGG
401 CCTTGGTCTT AGGCATCTCT TCTTTTGGGA TCACAGGAAT TCCTGCGACT
451 TTGCAATAGT TGCTTCGACA GAATCTATCT TTCCAAAAAC GCTCTATAGC
35 501 ATCGGAGAGC TTCTTTTAA AGATCGATAG TGCCCCCTCA GATGCCTCTG
551 TTTTATTATA AGCGTGCTT TTCCGCGGAG AGACTGCGAT CGTGGATGCG
601 TTAAGCCAAT TATTTGCCCA GCTCGATCTT TCTCCTAAAA AAATTTATCTT
651 TCTAGGAGAA GACCCTGAGG TCGTTCAAGC TGTTGGGTCT GCTTGATATG
701 GTTGGGGCAT GAACTTTTTA GGCTGGTAT ACTATCCTGC TCAAGAAAGC
40 751 CTTTTTCTT ATGTTTCATCC TTAATCTACA GCAACGAGC TCCAAGAAGC
801 ACAGGTTTA CAAGTAATTT CAGATGAAGT CGCACAGCTT ACTTTAAACG
851 CTCTCCGAA AATGAATTAA

```

The PSORT algorithm predicts inner membrane (0.123).

45 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 92A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 92B) and for FACS analysis.

These experiments show that cp7408 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 93

The following *C.pneumoniae* protein (PID 4376424) was expressed <SEQ ID 185; cp6424>:

```

1  MMHNIVVLSE  EPGRSAFLGR  TAFFPNKYPI  AQGGVGIPST  IGNLFTIWYC
5  51  FYFYRAATPQ  SDHPDGCIFI  LLERLKLGA  GFFYCDLRES  NTTGFTLFFE
101 GSNKGVLLKH  LFIRDE*

```

The cp6424 nucleotide sequence <SEQ ID 186> is:

```

1  ATGATGCACA  ATATTGTTGT  TCTTAGTGAG  GAACCTGGAC  GAAGCGCTTT
10  51  TCTTGGTAGG  ACGGCATTTT  TCCCTAATAA  GTATCCAATA  GCTCAGGGTG
101 GTGTGGAAT  ACCATCTACA  ATAGGCAATC  TCTTTACTAT  ATGGTACTGT
151 TTCTATTTT  ATAGAGCTGC  AACTCCACAA  TCTGATCATC  CTGACGGATG
201 TGGCTTTAT  CTACTAGAAA  GGCTTAAGGA  GCTCGGTGCA  GGGTTCTTTT
251 ATTGATGAT  TCGTGAGTCC  AATACCACTG  GCTTTACTCT  TTTTGTGAA
301 GGCTCCAATA  AAGGTGTGTT  AAAGAATCAC  TTGTTTATTA  GAGATGAGTA
351 A

```

15 The PSORT algorithm predicts cytoplasm (0.2502).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 93A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figure 93B) and for FACS analyses (Figure 93C; GST-fusion).

20 These experiments show that cp6424 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 94

The following *C.pneumoniae* protein (PID 4376449) was expressed <SEQ ID 187; cp6449>:

```

1  VASETYPYQI  LHAQREVRDA  YFNQADCHPA  RANQILEARK  ICLLDVYHTN
25  51  HYSVPTFCVD  NYPNLRFTFV  SSKNEMENGL  SNPLDNVLVE  AMVRRTHARN
101 LLAACKIRNI  EVPRVVGDL  RSGILISKLE  LKQPQFQSLT  EDFVNHSTNQ
151 EEARVHQKHV  LLISLILLCK  QAVLESFQEK  KRSS*

```

The cp6449 nucleotide sequence <SEQ ID 188> is:

```

1  GTGGCGTCTG  AAACGTATCC  TTCTCAGATA  TTGCACGCTC  AGAGGGAAGT
30  51  ACGTGATGCC  TATTTTAATC  AAGCGGATTG  CCATCCTGCT  CGGGCTAATC
101 AGATTCTCGA  GGCTAAGAAA  ATCTGTTTAT  TAGATGTTTA  TCATACTAAT
151 CATTATTCG  TATTTACTTT  TTGTGTAGAT  AATTATCCGA  ATCTCCGCTT
201 TACATTTGTA  TCTTCAAAAA  ACAATGAGAT  GAATGGCTTA  TCTAATCCTC
251 TAGATAATGT  TCTTGTAGAG  GCTATGGTAC  GTAGAACACA  TGCAAGAAAC
35  301  CTACTTGCAG  CGTGTAATAA  TCGAAATATT  GAGGTTCCAA  GGGTTGTTGG
351 GCTTGACCTA  AGATCTGGGA  TACTCATTTT  GAAACTAGAA  TTGAAGCAAC
401 CTCAGTTCCA  AAGTTTAACA  GAAGACTTCG  TAAATCATTC  CACAAATCAG
451 GAAGAAGCTC  GCGTCCATCA  AAAGCATGTG  TTGCTAATTT  CTTTAATTTT
501 ACTTTGCAAG  CAGGCCGTTC  TGAATCATT  CCAGGAAAAA  AAGCGATCCT
551 CTAA

```

40 The PSORT algorithm predicts inner membrane (0.2084).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 94A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figure 94B) and for FACS analyses (Figure 94C; GST-fusion).

45 These experiments show that cp6449 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 95

The following *C.pneumoniae* protein (PID 4376495) was expressed <SEQ ID 189; cp6495>:

MRELNAFELTQPEEYRNRWVLMPCCLKRCRFTQHAKVWSYRCVHEASLYEKNCFLLTYDDKHLPOYGSVLVHLHLQLFLKR
LRKMISPHKIRYFECGAYGFKLQRPYHLLLS

5 The cp6495 nucleotide sequence <SEQ ID 190> is:

TTGCGAGAATTAAGTCTTTGAATTAACCTCAACCTGAAGAGTATCGAAACCGTTGGGTTTGTATGCCTTGCTTAAGTGT
CGTTTTGTAGAACGCAACATGCAAAAGTCTGGTCTTATCGTTGTGTCCATGAAGCTTCTTTGTATGAGAAAAATTGTTTT
CTTACTTTGACTTATGATGATAAGCAATTTACCTCAGTATGGTTCGTTGGTAAAGCTGCATTTACAGCTGTTCTTAAGAGA
TTAAGAAAGATGATTTCTCTCATAAATTCGTTATTTGAATGTGGTTCGTTGGTAAACCAAATTACAAAGACCTCATTAT
CATCTACTTTTATCATGA

10 The PSORT algorithm predicts cytoplasmic (0.280).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 95A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 95B) and for FACS analysis (Figure 95C).

15 These experiments show that cp6495 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 96

The following *C.pneumoniae* protein (PID 4376506) was expressed <SEQ ID 191; cp6506>:

1 MRRFLFLILS SLPLVAFSAD NPTILEEKQS PLSRVSIIFA LPGVTPVSPD
51 GNCPIPWFSH SKKTLLEGQRI YYSGDSFGKY FVVSALWPNK VSSAVVACNM
101 ILKHRVDLIL IIGSCYSRSQ DSRFGSVLVS KGYINYDADV RPPFFERFPI
151 DIKKSVFATS EVHREAILRG GEEFISTHKQ EIEELLKTHG YLKSTTKTEH
201 TLMBGLVATG ESPAMSRNYP LSLQKLYPEI HGFDSVSGAV SQVCYBYSIP
251 CLGVNILLPH PLESRSNEDW KHLQSEASKI YMDTLKSVL KELCSSH*

25 The cp6506 nucleotide sequence <SEQ ID 192> is:

1 ATGCGTCGTT TTCTGTTTCT TATTCTTAGC TCTCTTCCTT TGGTCGCATT
51 CTCTGCTGAT AATTTCACTA TTCTAGAAGA AAAACAGAGT CCTTTAAGTC
101 GTGTAAGTAT TATTTTGGCT TTACCTGGGG TTAACCTCCGT TTCTTTTGAT
151 GGTAATTGTC CTATTCCTTG GTTTTCTCAT AGTAAAAAGA CTCTAGAGGG
30 201 ACAGAGAATT TATTACTCTG GCGACTCCTT TGGGAAATAC TTTGTAGTTT
251 CTGCTCTTTG GCCTAATAAA GTTCTTCAG CTGTTGTGGC TTGTAATATG
301 ATTCTTAAAC ATCGAGTGGG TCTTATTCTA ATTATAGGCT CGTGTACTC
351 TAGGTCTCAA GATAGCCGTT TTGGCAGCGT CTTAGTTTCT AAAGGCTACA
401 TTAATTATGA TGCAGATGTG AGGCCTTTCT TTGAAGATT TGAGATTCCA
35 451 GACATTAAAA AGAGTGTTTT TGCAACCACT GAGGTTTCATC GGGAGGCAAT
501 TCTTCGTGGA GGCGAAGAGT TTATTCTTAC CCATAAACAA GAAATCGAAG
551 AGCTTTTGAA GACTCATGGG TATTTGAAAT CAACAACCAA AACGGAGCAC
601 ACCTTAATGG AAGGTTTGGT TGCTACAGGC GAGTCTTTCT CGATGTCGCG
651 AAATATTTT CTTTCCCTTAC AAAAATGTGA TCCAGAGATT CATGGTTTGT
40 701 ATAGTGTGAG CGGCGCTGTT TCTCAGGTAT GCTATGAATA TAGCATTCCT
751 TGTTTAGGTG TGAATATCCT TCTCCCTCAT CCTTTAGAAAT CACGGAGTAA
801 CGAGGATTGG AAGCATCTTC AAAGTGAGGC AAGTAAATTT TATATGGATA
851 CCTTCTCAA GAGTGTATTA AAAGAACTCT GTTCTTCTCA TTAA

The PSORT algorithm predicts periplasmic space (0.571).

45 The protein was expressed in *E.coli* and purified as his-tag (Figure 96A) and GST-fusion (Figure 96B) products. The GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 96C) and for FACS analysis (Figure 96D).

These experiments show that cp6506 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 97

The following *C.pneumoniae* protein (PID 4376882) was expressed <SEQ ID 193; cp6882>:

```

5      1  MSLNLNPSSQ DSASEDSTSQ SQIFDPIRNR ELVSTPEEKV RQRLLSFLMH
      51  KLNYPKKLII IEKELKTLFP LLMRKGTLP KRRPDILII PPTYTDAQGN
     101  THNLGDPKPL LLIECKALAV NQNALKQLLS YNYSIGATCI AMAGKHSQVS
     151  ALFNPKTQTL DFYPGLPEYS QLLNYFISLN L*

```

The cp6882 nucleotide sequence <SEQ ID 194> is:

```

10      1  ATGTCCTTAT TGAACCTTCC CTCAAGCCAG GATTCTGCAT CTGAGGACTC
      51  CACATCGCAA TCTCAAATCT TCGATCCCAT TAGAAATCGG GAGTTAGTTT
     101  CTACTCCCGA AGAAAAAGTC CGCCAAAGGT TGCTCTCCTT CCTAATGCAT
     151  AAGCTGAAC TACCCTAAGAA ACTCATCATC ATAGAAAAAG AACTCAAAAC
     201  TCTTTTTCCT CTGCTTATGC GTAAAGGAAC CCTAATCCCA AAACGCCGCC
15      251  CAGATATTCT CATCATCACT CCCCCACAT ACACAGACGC ACAGGGAAAC
      301  ACTCACAAAC TAGGCGACCC AAAACCCCTG CTACTTATCG AATGTAAGGC
     351  CTTAGCCGTA AACCAAAATG CACTCAAACA ACTCCTTAGC TATAACTACT
     401  CTATCGGAGC CACCTGCATT GCTATGGCAG GGAACACTC TCAAGTGTCA
     451  GCTCTCTTCA ATCCAAAAC ACAAACTCTT GATTTTATC CTGGCCTCCC
20      501  AGAGTATTCC CAACTCCTAA ACTACTTTAT TTCTTTAAAC TTATAG

```

The PSORT algorithm predicts cytoplasm (0.362).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 97A). The protein was used to immunise mice, whose sera were used in a Western blot (Figure 97B) and for FACS analysis (Figure 97C).

25 These experiments show that cp6882 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 98

The following *C.pneumoniae* protein (PID 4376979) was expressed <SEQ ID 195; cp6979>:

```

30      1  MSVNPSPGNSK NDLWITGAHD QHPDVKESGV TSANLGSHRV TASGGRQGLL
      51  ARIKEAVTGF FSRMSFFRSQ APRGSQQPSA PSADTVRSPL PGGDARATEG
     101  AGRNLKKGY QPGMKVTIPQ VPGGGAQRSS GSTTLKPTRP APPPPKTGGT
     151  NAKRPATHGK GPAPQPKTG GTNAKRAATH GKGPAPQPPK GILKQPGQSG
     201  TSGKKRVSW DED*

```

The cp6979 nucleotide sequence <SEQ ID 196> is:

```

35      1  ATGTCGTGTA ATCCATCAGG AAATTCCTAAG AACGATCTCT GGATTACGGG
      51  AGCTCATGAT CAGCATCCCG ATGTTAAAGA ATCCGGGGTT ACAAGTGCTA
     101  ACCTAGGAAG TCATAGAGTG ACTGCCCTCAG GAGGACGCCA AGGGTTATTA
     151  GCACGAATCA AAGAAGCAGT AACCAGGTTT TTTAGTCGGA TGAGCTTCTT
     201  CAGATCGGGA GCTCCAAGAG GTAGCCAACA ACCCTCTGCT CCATCTGCAG
40      251  ATACTGTACG TAGCCCGTTG CCGGGAGGGG ATGCTCGCGC TACCGAGGGA
      301  GCTGTGAGGA ACTTAATTA AAAAGGGTAC CAACCAGGGA TGAAAGTCAC
     351  TATCCACACAG GTTCCTGGAG GAGGGGCCCA ACGTTCATCA GGTAGCACGA
     401  CACTAAAGCC TACGCGTCCG GCACCCACAC CTCCTAAAAC GGGTGGAAGT
     451  AATGCAAAAC GTCCGGCAAC GCACGGGAAG GGTCCAGCAC CCCAGCCTCC
45      501  TAAACAGGT GGGACCAATG CTAAGCGCGC AGCAACGCAT GGGAAAGGTC
      551  CAGCACCTCA ACCTCCTAAG GGCATTTTGA AACAGCCTGG GCAGTCTGGG
     601  ACTTCAGGAA AGAAGCGTGT CAGCTGGTCT GACGAAGATT AA

```

The PSORT algorithm predicts cytoplasm (0.360).

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The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 98A). The GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 98B) and for FACS analysis (Figure 98C).

These experiments show that cp6979 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 99

The following *C.pneumoniae* protein (PID 4377028) was expressed <SEQ ID 197; cp7028>:

```

1  MLLGFLCDCP CASWQCAAVA NCYDSVFMSR PEHKPNIPYI TKATRRGLRM
51  KTLAYLASLK DARQLAYDFL KDPGSLARLA KALIAPKEAL QEGNLPFYGC
101 SNIEDILEEM RRPHRILLIG FSYCQPKAC PEGRFNDACR YDPSHPTCAS
151 CSIGTMMRLN ARRYTTVIIP TFIDIAKHLH TLKKRYPGYQ ILFAVTACEL
201 SLKMFPGDYAS VMNLKGVGIR LTGRICNTFK AFKLAERGVK PGVTILEEDG
251 FEVLARILTE YSSAPFPRDF CEIH*
```

The cp7028 nucleotide sequence <SEQ ID 198> is:

```

1  ATGCTTCTAG GGTTTTGTG TGA CTGCCCC TGTGCTTCGT GGCAGTGTGC
51  GGCCGTTGCT AATTGTTATG ATTCCGTATT TATGCTAGA CCAGAGCACA
101 AACCTAATAT TCCTTATATT ACTAAAGCTA CAAGACGGGG TCTGCGTATG
151 AAGACGCTTG CTTATCTGGC CTCTTTAAAA GATGCTAGAC AGCTTGCCTA
201 TGATTCTCTG AAAGATCCTG GTTCTTTAGC TCGGTTAGCT AAGGCTTTGA
251 TAGTCCTTAA GGAGGCCTTA CAGGAGGGCA ACCTATTTTT TTATGGCTGT
301 AGTAATATTG AGGATATTTT AGAGGAGATG CGTCGTCCTC ATAGAATCCT
351 TTTGTTAGGA TTTTCTTATT GTCAAAAGCC TAAGGCATGT CCTGAAGGGC
401 GTTTCATAGA TGCTTGTCGG TATGATCCTT CACATCCTAC ATGTGCCTCA
451 TGTTCATAGG GGACCATGAT GCGGCTGAAT GCTCGTAGAT ACACTACTGT
501 GATCATCCCT ACATTTATAG ATATCGCAAA ACATTTACAC ACTTTAAAAA
551 AGCGCTACCC TGGATATCAA ATTCTCTTTG CAGTTACTGC TTGTGAACTT
601 TCCTTAAAAA TGTTTGGAGA TTATGCCTCC GTAATGAACT TAAAGGGTGT
651 GGGCATCAGA CTCACAGGAC GTATTTCGCA TACATTTAAG GCATTTAAAT
701 TAGCTGAGCG AGGAGTCAAA CCAGGAGTCA CTATCCTAGA AGAAGATGGC
751 TTTGAGGTAT TAGCAAGGAT TCTTACAGAA TACAGTAGCG CTCCTTTCCC
801 TAGAGACTTT TGTGAGATCC ATTAG
```

The PSORT algorithm predicts cytoplasm (0.1453).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 99A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 99B) and for FACS analysis (Figure 99C).

These experiments show that cp7028 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 100

The following *C.pneumoniae* protein (PID 4377355) was expressed <SEQ ID 199; cp7355>:

```

1  MKKVVTLSII FFATYCASEL SAVTVVAVPL SEAPGKIQVR PVVGLQFQRE
51  QGSVPYSFYY PYDYGYYYPE TYGYTKNTGQ ESRECYTRFE DGTIFYECD*
```

The cp7355 nucleotide sequence <SEQ ID 200> is:

```

1  ATGAAGAAAG TCGTAACACT ATCCATTATA TTTTTCGCAA CGTATTGTGC
51  ATCAGAGCTT AGTGCTGTAA CTGTAGTGGC TGTGCCTTTA TCAGAGGCTC
101 CAGGGAAGAT TCAAGTTCGT CCCGTCGTTG GTCTGCAATT TCAAGAAGAA
151 CAGGGTTCGT TGCCCTATAG TTTTATTAT CCTTATGACT ATGGGTATTA
201 CTATCCAGAG ACTTATGGCT ATACTAAAAA TACAGGTCAA GAAAGTCGCG
```

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251 AATGTTATAC CCGATTGAA GATGGCACAA TTTTATGA ATGCGATTAG

The PSORT algorithm predicts inner membrane (0.143).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 100A) and a his-tag product. The proteins were used to immunise mice, whose sera were used in a Western blot (Figure 100B) and for FACS analysis (Figure 100C).

These experiments show that cp7355 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 101

The following *C.pneumoniae* protein (PID 4377380) was expressed <SEQ ID 201; cp7380>:

```

10      1  VHVCERTLDP  KYILKIALKL  RQSLSLFFQN  SQSLQRAYST  PYSYVRIILQ
      51  KENKEKQALA  RHKCISILEF  FKNLLFVHLL  SLSKNQREGC  STDMAVVSTP
     101  FFNRNLWYRL  LSSRFSWLKS  YCPRFFLDYL  EAFGLLSDFL  DHQAVIKFFE
     151  LETHFSYYPV  SGFVAPHQYL  SLLQDRYFPI  ASVMRTLDDK  NFSLTPDLIH
     201  DLLGHVPWLL  HPSFSEFFIN  MGRLPKIVIE  KVQALPSKKQ  RIQTQSNLI
     251  AIVRCFWFTV  RSGLIENHEG  RKAYGAVLIS  SPQELGHAFI  DNVRVLPLEL
     301  DQIIRLPFNT  STPQETLFSI  RHFDELVELT  SKLEWMLDQG  LLESIPLYNQ
     351  EKYLSGFEVL  CQ*
```

The cp7380 nucleotide sequence <SEQ ID 202> is:

```

20      1  GTGCACTACT  GCGAGAGAAC  CCTGGACCCA  AAGTATATTC  TGAAGATTGC
      51  TCTAAAGCTG  AGACAATCAC  TTTCCCTGTT  CTTCCAGAAC  AGCCAATCAC
     101  TCCAACGTGC  ATACTCGACC  CCATATTCC  ACTACCGAAT  CATTCTACAA
     151  AAGGAAAATA  AAGAGAAGCA  AGCTTTAGCT  CGACACAAAT  GCATTCTAT
     201  TTTAGAATTT  TTCAAAAAC  TACTCTTTGT  TCATCTTCTG  TCATTATCAA
     251  AGAATCAAAG  GGAAGGTTGC  TCCACTGATA  TGGCTGTTGT  AAGCACTCCC
     301  TTTTTTAATC  GGAATTTATG  GTATCGACTC  CTTTCCTCAC  GGTTTTCTCT
     351  ATGGAAAAGC  TATTGTCCAA  GATTTTTCCT  TGATTACTTA  GAAGCTTTCG
     401  GTCTCCTTTC  TGATTTCCTA  GACCATCAAG  CAGTCATTAA  ATTCTTCGAA
     451  TTAGAAACAC  ATTTTTCCTA  TTATCCCGTT  TCAGGATTGT  TAGCTCCCCA
     501  TCAATACTTG  TCTCTGTTGC  AGGACCGTTA  CTTTCCCAT  GCCTCTGTAA
     551  TGCGAACTCT  CGATAAAGAT  AATTTCCTCT  TAACCTCTGA  TCTCATCCAT
     601  GACCTTTTAG  GGCACGTGCC  TTGGCTTCTA  CATCCCTCAT  TTTCTGAATT
     651  TTTCATAAAC  ATGGGAAGAC  TCTTCACTAA  AGTCATAGAA  AAAGTACAAG
     701  CTC'TCCTAG  TAAAAACAA  CGCATACAAA  CCTACAAAG  CAATCTGATC
     751  GCTATTGTAC  GCTGCTTTTG  GTTTACTGTT  GAAAGCGGAC  TTATTGAAAA
     801  CCATGAAGGA  AGAAAAGCAT  ATGGAGCCGT  TCTTATCAGT  TCTCCTCAGG
     851  AACTTGGACA  CGCTTTCATT  GATAACGTAC  GTGTTCTCCC  TTTAGAATTG
     901  GATCAGATTA  TTCGTCTTCC  CTTCAATACA  TCAACTCCAC  AAGAGACTTT
     951  ATTTTCAATA  AGACATTTTG  ATGAACTGGT  AGAACTCACT  TCAAAATTAG
    1001  AATGGATGCT  CGACCAAGGT  CTGTTAGAA  CAATTCCCCT  TTACAATCAA
    1051  GAGAAATATC  TTTCTGGTTT  TGAGGTACTT  TGCCAATGA
```

The PSORT algorithm predicts inner membrane (0.1362).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 101A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 101B) and for FACS analysis (Figure 101C).

These experiments show that cp7380 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 102

The following *C.pneumoniae* protein (PID 4376904) was expressed <SEQ ID 203; cp6904>:

-135-

```

1  MMNYEDAKLR  GQAVAILYQI  GAIKFGKHIL  ASGEETPLYV  DMRLVISSPE
51 VLQTVATLIW  RLRPSFNSSL  LCGVPYTALT  LATSISLKYN  IPMVLRRKEL
101 QNVDPDAIK  VEGLFPPGQT  CLVINDMVSS  GKSIETAVA  LEENGLVVRE
151 ALVFLDRRKE  ACQPLGPQGI  KVSSVFTVPT  LIKALIAYGK  LSSGDLTLAN
201 KISEILEIES  *

```

The cp6904 nucleotide sequence <SEQ ID 204> is:

```

1  ATGATGAACT  ACGAAGATGC  AAAATTACGC  GGTCAAGCTG  TAGCAATTCT
51 ATACCAAATC  GGAGCTATAA  AGTTCGGAAA  ACATATTCTC  GCTAGCGGAG
101 AAGAAACTCC  TCTGTATGTA  GATATGCGTC  TTGTGATCTC  CTCTCCAGAA
151 GTTCTCCAGA  CAGTGGCAAC  TCTTATTTGG  CGCCTCCGCC  CCTCATTCAA
201 TAGTAGCTTA  CTCTGCGGAG  TCCCTTATAC  TGCTCTAACC  CTAGCAACCT
251 CGATCTCTTT  AAAATATAAC  ATCCCTATGG  TATTGCGAAG  GAAGGAATTA
301 CAGAATGTAG  ACCCCTCGGA  CGCTATTAAA  GTAGAAGGGT  TATTACTTCC
351 AGGACAAACT  TGTTTAGTCA  TCAATGATAT  GGTTCCTCA  GGAAAATCTA
401 TAATAGAGAC  AGCAGTCGCA  CTGGAAGAAA  ATGGTCTGGT  AGTTCGTGAA
451 GCATTGGTAT  TCTTAGATCG  TAGAAAAGAA  GCGTGTCAAC  CACTTGGTCC
501 ACAGGGAATA  AAAGTCAGTT  CGGTATTTAC  TGTACCCACT  CTGATAAAAG
551 CTTTGATCGC  TTATGGGAAG  CTAAGCAGTG  GTGATCTAAC  CCTGGCAAAC
601 AAAATTCCG  AAATCTAGA  AATTGAATCT  TAA

```

The PSORT algorithm predicts cytoplasm (0.0358).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 102A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 102B) and for FACS analysis.

The cp6904 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp6904 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 103

The following *C.pneumoniae* protein (PID 4376964) was expressed <SEQ ID 205; cp6964>:

```

1  MKKLIALIGI  FLVPIKGNIN  KEHDAHATVL  KAARAKYNLF  FVQDVFPVHE
51 VIEPISPDCL  VHYEGWV*

```

The cp6964 nucleotide sequence <SEQ ID 206> is:

```

1  ATGAAAAAAT  TGATTGCTTT  GATAGGGATA  TTTCTTGTTT  CAATAAAAGG
51 AAATACCAAT  AAGGAACACG  ACGCTCACGC  GACTGTTTAA  AAAGCGGCCA
101 GAGCAAAGTA  TAATTGTGTT  TTTGTTTCA  ATGTTTTCCT  TGTACACGAA
151 GTTATCGAGC  CTATTTCTCC  CGATTGCCTG  GTACATTATG  AAGGGTGGGT
201 TTGA

```

The PSORT algorithm predicts inner membrane (0.091).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 103A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 103B) and for FACS analysis (Figure 103C).

These experiments show that cp6964 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 104

The following *C.pneumoniae* protein (PID 4377387) was expressed <SEQ ID 207; cp7387>:

-136-

```

1  LNFPAKIDHNH LYLTCGLDGLG VACPILSTDC LPNYSEKASH EVLVYSKFRG
51  ISGEPSRLAT SGNDYYYSIV SLPGLRYEV TSPSGRHDFN IDMHVAPKIG
101 AVLSHGTREA KEIPGSSKDY AFFSLTARES LMISEKLAMT FQVSEVIQNC
151 YSQCTKVTKT NLKEQYRHLN HNTGFELSVK SAF*

```

5 The cp7387 nucleotide sequence <SEQ ID 208> is:

```

1  TTGAATTTTG CAAAGATTGA TCACAATCAT CTCTACCTTA CATGTTTGGG
51  AGATCTTGGT GTAGCTTGTC CTATACTTTC TACAGATTGT CTACCTAATT
101 ATAGCGAGAA AGCATCTCAT GAGGTTCTTG TTTATAGTAA ATTTAGATGC
151 ATTTCTGGAG AGCCATCTCG ACTTGCAACT TCAGGAAATG ACACATATTA
10  201 TTCTATAGTA AGTTTACCTA TAGGACTCCG TTACGAAAGT ACTTCACCAT
251 CAGGACGTCA TGATTTCAT ATTGATATGC ATGTAGCTCC AAAGATAGGT
301 GCAGTACTCT CTCATGGAAC ACGAGAGGCT AAAGAGATCC CAGGATCTTC
351 AAAAGACTAT GCATTTTTTA GCTTGACTGC TAGAGAAAGT TTAATGATTT
401 CTGAAAAGCT TGGGATGACT TTCCAAGTTA GCGAAGTTAT TCAGAATTGT
15  451 TATTCACAAT GTACTAAAGT AACGAAAACG AATTTAAAG AACAGTATAG
501 GCACTTATCC CACAATACAG GGTTTGAGTT AAGCGTCAAG TCTGCATTCT
551 AA

```

The PSORT algorithm predicts inner membrane (0.043).

20 The protein was expressed in *E.coli* and purified as a his-tagged-fusion product (Figure 104A) and also as a GST-fusion (Figure 104B). The recombinant proteins were used to immunise mice, whose sera were used in a Western blot and for FACS analysis (Figure 104C; his-tagged).

These experiments show that cp7387 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 105

25 The following *C.pneumoniae* protein (PID 4376281) was expressed <SEQ ID 209; cp6281>:

```

1  MFLQFFHPIV FSDQSLSFLP YLGKSSGIIE KCSNIVEHYL HLGGDTSVII
51  TGVSGATFLS VDHALPISKS EKIIKILSYI LILPLILALF IKIVLRIILF
101 FKYRGLILDV KKEDLKKTLT PDQENLSLPL PSPPTLKKIH ALHILVRSGK
151 TYNELIQEGF SFTKITDLGQ APSPKQDIGF SYNSSLPNFY FHSLSVSPNI
30  201 SGEERALNYH KBQOEEMAVK LRTMQACSFV FRSLHLPSMQ TKDKKAGFGL
251 LITFFPWKIYP L*

```

The cp6281 nucleotide sequence <SEQ ID 210> is:

```

1  ATGTTTCTTC AGTTTFTTCA TCCTATAGTC TTCTCGGATC AGTCCTTATC
51  TTTTCTTCCT TACCTAGGAA AAAGCTCTGG CATTATTGAA AAATGTTCCA
35  101 ATATCGTTGA ACACTATTTA CATTTGGGAG GAGACACTTC TGTATCATC
151 ACAGGAGTTT CTGGAGCTAC CTTTCTATCT GTTGATCATG CCCTCCCAAT
201 CTCGAAATCT GAAAAAATAA TAAAAATTCT CTCCTATATT TTAATCTTTC
251 CTCTGATTCT AGCTCTCTTT ATTAAGATCG TTTTACGCAT TATCTTATTC
40  301 TTCAAGTATC GTGGTCTAAT CCTAGATGTT AAGAAGGAGG ATTTGAAAAA
351 AACACTTACA CCTGACCAAG AAAACCTCAG TCTTCTTTTA CCATCTCCTA
401 CAACATTAAG GAAAAATCAT GCGCTACACA TTTTAGTGCG TTCTGGAAAA
451 ACCTATAACG AGCTTATACA AGAAGGGTTT TCTTTCACTA AAATCACAGA
501 TCTTGGTCAA GCTCTTTCAC CAAAGCAAGA TATTGGCTTC TCTTATAATT
551 CCTTCTCCTC TAACTTCTAT TTTTCATTCCT TGGTATCTGT TCCAAATATT
45  601 TCAGGCGAGG AACGGGCTCT TAATTATCAT AAAGAACAAC AAGAGGAAAT
651 GGCTGTTAAA TAAAAACAA TGCAAGCGTG TTCTTTTGTC TTCCGATCCC
701 TGCATTTACC TTCAATGCAA ACGAAGGACA AAAAGGCTGG ATTTGGACTA
751 CTGACGTTTT TCCCTTGGAA AATCTACCCC CTATAA

```

The PSORT algorithm predicts inner membrane (0.5373).

50 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 105A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 105B) and for FACS analysis.

These experiments show that cp6281 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 106 and Example 107

- 5 The following *C.pneumoniae* protein (PID 4376306) was expressed <SEQ ID 211; cp6306>:

```

1  MGNHETYIHP  GVLPSASHAQD  VSRSTVYPSR  SFIMRRMLMG  WNFNRVPSKS
51  SEQLMDGHRI  PLIFFGKHPH  TISILNVNRF  SWLSIFYNGE  RGF*
```

The cp6306 nucleotide sequence <SEQ ID 212> is:

```

1  ATGGGAAACC  ATGAGACCTA  TATACATCCA  GGAGTGCTCC  CGAGTAGTCA
10  51  TGCTCAGGAT  GTTAGCAGAT  CTACAGTTTA  CCCAGTCGA  AGTTTATCA
    101  TGAGACGTAT  GCTCATGGGC  TGGAAATTCA  ATCGTGTTC  CTCGAAGAGC
    151  TCCGAGCAGT  TAATGGATGG  TCATCGCATA  CCTCTTATAT  TTTTGGGAA
    201  GCATCATCCT  ACTATATCTA  TTTTAAATGT  CAATAGATTT  TCTTGGCTCT
    251  CCATTTTTTA  CAATGGAGAA  AGGGGGTTTT  GA
```

- 15 The PSORT algorithm predicts cytoplasm (0.167).

The following *C.pneumoniae* protein (PID 4376434) was also expressed <SEQ ID 213; cp6434>:

```

1  MSESINRSIH  LEASTPFFIK  LTNLCSRLV  KITSLVISLL  ALVGAGVTLV
51  VLFVAGILPL  LPVLILBIL  ITVLVLLFCL  VLEPYLIEKP  SKIKELPKVD
101  ELSVVFETDST  L*
```

- 20 The cp6434 nucleotide sequence <SEQ ID 214> is:

```

1  ATGTCTGAAA  GTATTAACAG  AAGCATTCAT  TTAGAAGCCT  CTACACCATT
25  51  TTTTATAAAA  TTAACGAATC  TCTGTGAAAG  TAGATTAGTT  AAGATCACTT
    101  CTCTTGTTAT  TTCTCTATTA  GCTTTAGTGG  GTGCGGGAGT  CACTCTTGTC
    151  GTTTTATTTG  TAGCTGGGAT  CCTTCCTTTA  CTTCTGTAC  TCATCTTAGA
    201  AATTATTTTA  ATAACCGTCC  TTGCTTGCT  TTTTGTGTTG  GTATTGGAAC
    251  CTTATTTAAT  AGAAAAACCT  AGTAAATAA  AGGAAGTACC  TAAAGTAGAC
    301  GAGCTATCTG  TAGTAGAAAC  GGACAGTACT  CTTTAA
```

The PSORT algorithm predicts inner membrane (0.6859).

- 30 The proteins were expressed in *E.coli* and purified as his-tag products (Figure 106A; 6306 = lanes 2-4; 6434 = lanes 8-10). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 106B & 107) and for FACS analysis.

These experiments show that cp6306 & cp6434 are surface-exposed and immunoaccessible proteins, and that they are useful immunogens. These properties are not evident from the sequences alone.

Example 108

- 35 The following *C.pneumoniae* protein (PID 4377400) was expressed <SEQ ID 215; cp7400>:

```

1  MRVMRFFCLF  FLGFLGSFHC  VAEDKGVDLF  GVWDDNQITE  CDDSYMTEGR
51  EEVEKVVDAA
```

The cp7400 nucleotide sequence <SEQ ID 216> is:

```

40  1  GTGAGAGTTA  TGAGATTTTT  TTGTCTATTT  TTTCTTGGGT  TCCTAGGATC
    51  TTTTCATTGT  GTTGTGGAAG  ACAAGGGCGT  GGATTATTTT  GGAGTCTGGG
    101  ACGATAACCA  AATTACAGAG  TGTGACGATA  GTTACATGAC  AGAGGGTCGT
    151  GAAGAGGTTG  AAAAGGTAGT  GGACGCTTAG
```

The PSORT algorithm predicts periplasmic space (0.924).

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The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 108A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 108B) and for FACS analysis.

These experiments show that cp7400 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 109

The following *C.pneumoniae* protein (PID 4376395) was expressed <SEQ ID 217; cp6395>:

```

1  MENAMSSSFV YNGPSWILKT SVAQEVFKKH GKGIQVLLST SVMLEFIGLGV
51  CAFIFPQYLI VFVLTIALLM LAISLVLFLL IRSVRSSMVD RLWCSEKGYA
101 LHQHENGPFLL DVKRVQQILL RSPYIKVRAL WPSGDIPEDP SQAAVLLLSLSP
151 WTTFFSSVDVE ALLPSPQKKK GKVIDPVLPK LSRIERVSLV VFLSAFTLDD
201 LNEQGVNPLM NNEEFLEFFIN KKAREHGIQD LKHEIMSSLE KTGVPPLDPSM
251 SFQVSQAMFS VYRYLRQRDL TTSELRCFHL LSCFRGDVVH CLASFENPKD
301 LADSDPLEAC KNVEWGEFIS ACEKALLKNP QGISIKDLKQ FLVR*

```

The cp6395 nucleotide sequence <SEQ ID 218> is:

```

1  ATGGAGAATG CTATGTCATC ATCGTTTGTG TATAATGGGC CTCGTGGAT
51  TTTAAAAACG TCAGTAGCTC AGGAGGTATT TAAAAAGCAC GGTAAGGGGA
101 TTCAGGTTCT CTTAAGTACT TCAGTGATGC TTTTATAGG TCTTGGAGTC
151 TGTGCCTTTA TATTTCTCTA ATATCTGATT GTTTTGTGTT TGACTATAGC
201 TTTTGGCTTATG CTCGCTATAA GCTTGGTATT GTTCTCTTA ATACGTTCTG
251 TACGCTCTTC AATGGTAGAT CGTTTGTGGT GTTCTGAAAA AGGATATGCT
301 CTTTCATCAAC ATGAGAACGG GCCTTTTGTG GATGTGAAGC GTGTACAGCA
351 AATTCTTCTA AGATCACCCCT ATATTAAAGT TCGGGCTTTA TGGCCGTCCTG
401 GAGATATCCC TGAGGATCCT TCACAAGCTG CGGTTCTATT ACTTTCCTCT
451 TGGACTTTCT TTTCAATCCGT GGATGTAGAG GCTTTATTAC CGAGTCCTCA
501 AGAAAAGGAG GGTAAGTATA TAGATCCTGT GCTGCCTAAG TTGTCTAGGA
551 TAGAGAGAGT CTCACTTTTA GTGTTTGTGA GTGCATTAC TTTGGATGAC
601 TTAAACGAAC AGGGAGTCAA TCCTTTGATG AATAATGAGG AATTTTATT
651 TTTTATAAAT AAGAAAGCGC GTGAGCATGG GATTTCAGGAT TTAAACACG
701 AGATTATGTC TTCGTTAGAG AAAACAGGAG TGCCATTAGA CCCCTCAATG
751 AGTTTTC AAG TTTTCAAG TTTTCAAGC GATGTTTCT GTATATCGCT ACTTGAGACA
801 AAGGGATTTA ACGACTTCAG AATTAAGATG TTTTCACTC TTAAGTTGTT
851 TTAAAGGGGA TGTGGTTCAT TGTTTAGCTT CATTGAAAA CCCTAAAGAT
901 TTAGCAGATT CTGACTTTT AGAAGCTTGT AAGAACGTGG AATGGGGTGA
951 GTTTATTTTCG GCATGTGAGA AGGCTCTTTT AAAGAATCCG CAAGGAATTT
1001 CCATTAAAGGA TCTAAACAA TTTTGTAGTGA GGTAA

```

The PSORT algorithm predicts inner membrane (0.6307).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 109A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 109B) and for FACS analysis.

These experiments show that cp6395 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 110

The following *C.pneumoniae* protein (PID 4376396) was expressed <SEQ ID 219; cp6396>:

```

1  MIEFAFVPHT SVTADRIEDR MACRMNKLST LAITSLCVLI SSVCMIGIL
51  CISGTVGTYA FVVGIIFSVL ALVACVFFLY FFYFSSEKFK CASSQEFRLF
101 PIPAVVSALR SYEYISQDAI NDVIKDTMQL STLSSLLDPE APFLEFPYFN
151 SLIVNHSMEKE ADRLSREAFI ILLGEITWKD CETKILEPWLK DPNITPDDEFW
201 KLLKDHFDLK DFKKRIATWI RKAYPEIRLP KKHCLDKSIY KGCCKFLLLS

```

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251 ENDVQYQRLH HKVCYFSGEF PAMVLGLGSE VPMVLGLPKV PKDLTWEMFM
301 ENMPVLLQSK REGHWKISLE DVASL*

The cp6396 nucleotide sequence <SEQ ID 220> is:

```

5      1  ATGATCGACT TTGCTTTTGT TCCTCATACC TCCGTGACAG CGGATCGGAT
      51  TGAGGATCGC ATGGCCTGTC GCATGAACAA GTTGTCTACT TTAGCAATTA
101    101  CAAGTCTTTG TGTATTGATC AGTTCAGTTT GTATTATGAT TGGGATTTTA
      151  TGCATTTCTG GAACGGTTGG GACCTATGCA TTTGTGTAG GAATTATTTT
201    201  TTCTGTGCTT GCTTTGGTAG CATGTGTTT CTTCTTTTAT TTCTTTTATT
      251  TTTCTTCTGA GGAATTTAAG TGTGCTTCTT CGCAGGAGTT TCGTTTTTTG
10     301  CCTATACCAG CTGTGGTTTC TGCATTGCGT TCCTATGAAT ACATTTCTCA
      351  GGACGCTATC AATGACGTTA TAAAAGATAC GATGCAGTTG TCTACCCTTT
401    401  CTTCTCTTTT AGATCCCGAA GCTTTTTTCT TAGAATTTCC TTATTTTAAC
      451  TCTTTGATAG TGAATCATTC GATGAAGGAA GCGGATCGTT TGTCTCGAGA
15     501  GGCTTTTTTG ATTTTATTAG GTGAGATTAC TTGGAAGGAT TGTGAAACAA
      551  AAGATTTGCG ATGGTTGAAA GATCCTAATA TCACTCCTGA TGATTTCTGG
601    601  AAGCTATTAA AAGACCATTT CGATTTAAG GACTTTAAGA AGAGGATCGC
      651  CACTTGGATA CGGAAGGCCT ATCCAGAAAT TAGATTACCG AAGAAGCATF
701    701  GTTTAGATAA GTCTATCTAT AAGGGGTGTT GTAAGTTTTT ATTACTTTCT
20     751  GAGAATGATG TGCAATATCA GAGGTTATTA CATAAGGTCT GTATTCTCTC
      801  TGGGGAGTTT CCTGCCATGG TTTTAGGTTT GGGAAAGTAA GTGCCATGG
      851  TGTTAGGACT CCTAAGGTT CCAAGGATC TTACCTGGGA GATGTTTATG
901    901  GAAATATGTC CTGTTCTTCT GCAAAGCAA AGAGAGGGGC ATTGAAAAT
951    951  CTCCTTGAA GACGTAGCCT CTCTTTAA

```

The PSORT algorithm predicts inner membrane (0.6095).

- 25 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 110A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 110B) and for FACS analysis.

These experiments show that cp6396 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

30 Example 111

The following *C.pneumoniae* protein (PID 4376408) was expressed <SEQ ID 221; cp6408>:

```

35     1  MNTSLKRPLK SHFDVVSFL RPEHLKKTRE SLKEGSISLD QLMQIEDIAI
      51  QDLIKRQKAA GLSFTIDGEF RRATWHYDFM WGFHGVGHHR ATEGVFFDGE
101    101  RAMIDDTYLT DKISVSHHPF VDHFKFVKAL EDEFTTAKQT LPAPAQFLKQ
      151  MIFPNNIEVT RKFYPTNQEL IEDIVAGYRK VIRDLVDAGC RYLQLDDCTR
201    201  GGLVDPVRCV WYGIDEKGLQ DLIQQYLLIN NLVIADRPDD LVVNLHVCRG
      251  NYHSKFFASG SYDFIAKPLF EQTNVDGYL EFDHERSGDF SPLTFISGEK
301    301  TVCLGLVTSK TPTLENKDEV IARIHQAADY LPLERLSLSP QCGFASCEIG
351    351  NKLTREEQWA KVALVKEISE EVWK*

```

40 The cp6408 nucleotide sequence <SEQ ID 222> is:

```

45     1  ATGAATACTT CACTAAAAAG ACCTCTGAAA TCTCATTTTG ATGTTGTCGG
      51  TAGTTTTTTG CGTCCTGAGC ATTTAAAAAA AACTAGAGAA AGCCTTAAAG
101    101  AAGGCTCTAT TTCTCTAGAT CAACTCATGC AAATTGAGGA TATCGCTATC
      151  CAAGATTGTA TCAAAAAACA AAAAGCAGCA GGTCTTTCTT TTATTACTGA
201    201  TGGAGAATTC CGCAGAGCTA CGTGGCATTG CGACTTCATG TGGGGTTTTT
      251  ATGGCGTAGG TCACCACAGA GCTACAGAAG GAGTTTCTTT TGATGGAGAA
301    301  CGCGCTATGA TCGATGATAC CTATCTGACA GACAAGATCT CTGTATCTCA
      351  CCACCCATTT GTGGATCACT TTAAATTTGT AAAAGCTCTA GAAGATGAAT
50     401  TTACGACTGC AAAGCAAACCT CTTCTGACAC CGGCACAGTT TTTAAAGCAG
      451  ATGATCTTCC CTAATAATAT AGAGGTCACA CGTAAATTCT ATCCTACAAA
      501  TCAGGAGCTA ATTGAAGATA TTGTTGCAGG TTATCGTAAA GTCATTCGCG
      551  ATCTTTATGA TGCTGGCTGC CGCTATCTCC AATTAGATGA CTGTACTCGG
601    601  GGAGGTTTAG TAGACCCTCG AGTCTGTTTC TGGTATGGTA TCGATGAAAA
      651  AGGTCTTCAA GATCTGATTC AACAATATCT TCTGATTAAT AATCTTGTA
55     701  TTGCAGATCG TCCCGATGAT CTAGTCGTTA ATTTACATGT ATGCCGTGGG

```

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```

751  AACTACCACT  CAAAATTCTT  TGCTAGTGGT  AGTTATGACT  TTATTGCAAA
801  GCCCCTATTC  GAACAAACAA  ATGTAGACGG  CTACTATTTA  GAGTTTGATC
851  ATGAGCGTTC  TGGAGACTTC  TCTCCTCTCA  CCTTCATTTC  TGGAGAAAAA
901  ACTGTCTGCT  TAGGTCTTGT  TACCAGCAAA  ACCCCTACAC  TTGAAAATAA
951  GGATGAGGTC  ATTGCTCGCA  TACATCAAGC  AGCAGACTAC  CTGCCCTTGG
1001 AAAGACTCTC  TCTAAGTCCA  CAGTGTGGTT  TTGCTTCATG  TGAAATAGGA
1051 AATAAATTAA  CAGAAGAAGA  GCAATGGGCT  AAAGTTGCTC  TAGTAAAGA
1101 AATTTCGGAA  GAAGTTTGA  AATAA

```

The PSORT algorithm predicts cytoplasm (0.2171).

- 10 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 111A) and also as a his-tagged product. The his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 111B) and for FACS analysis.

These experiments show that cp6408 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

15 Example 112

The following *C.pneumoniae* protein (PID 4376430) was expressed <SEQ ID 223; cp6430>:

```

1  MKLYSISSDV  DTPWIFQLMS  KVDSYFLGG  NRIKVVSIWM  QEPNLIIGKV
51  ENVRISTIVK  ILKILSFLIF  PLILIALALH  YFLHAKYANH  LLVSKILERA
101 PQYVIPGRS  GDTASHYKLT  TLVPVSQKNL  QAMGSPNPLEV  EAALRTTKPS
151 FFCVPAKYRQ  IISSHGIRF  SLDLBQLADD  INLDSVSWPT  EYLNSTMDFC
201 SKADKRVIQN  VQNLRTGTYY  NSVGKRSLLK  FMLQHLFIDG  ITQENPEALP
251 NNTSGRLTLF  PSVRYIYSHF  TPQNPTIWPQ  VFFRQGPLDE  DRGGGFLEILE
301 QLQELGVRFP  ICPSQGPDPN  NFQGFQGIRI  YWEDSYQPNK  EV*

```

The cp6430 nucleotide sequence <SEQ ID 224> is:

```

25 1  ATGAAACTTT  ATAGCATCTC  TTCAGATGTA  GATACACCTT  GGATATTTCA
51  GCTTATGTCA  AAGGTAGATT  CTTATCTTTT  CTTAGGCGGG  AATAGAATCA
101 AGGTTGTATC  TATAGTTATG  CAAGAACCTA  ACTTAATTAT  TGGAAAAGTA
151 GAAACGTTTC  GGATCTCCAC  AATAGTGAAG  ATATTAAAGA  TTTTATCCTT
201 CTTAATCTTC  CCTCTGATTT  TAATCGCTTT  AGCCCTACAC  TATTTTCTAC
30 251 ATGCTAAATA  TGCTAATCAC  TTAATGTAT  CTAAGATTTT  AGAAAGAGCT
301 CCTCAGTATG  TGCCTATTCC  TGGTCGTTC  GGAGACACGG  CGTCTCATT
351 TAAATTAACA  ACATTGGTTC  CAGTATCCCA  AAAAAATCTA  CAAGCTATGG
401 GATCAAAATC  TCTAGAAGTT  GAAGCGGCTC  TTCGAACTAC  AAAACCCTCT
451 TTTTCTGTG  TACCTGCAAA  ATACCGTCAG  ATTATAATTT  CAAGTCACGG
35 501 CATTGCTTTT  TCTTTAGATC  TTGAACAAC  TGCTGATGAC  ATTAATTTAG
551 ATTCGGTTTC  CTGGCTACG  GAGTATCTTA  ACTCTACTAT  GGATTTTTC
601 AGCAAGCAG  ATAAACGTGT  TATACAGAAT  GTACAAAATC  TCGGACAGG
651 AACTTACATA  AATTCTGTAG  GAAAGCGTAG  CCTTTTAAAA  TTCATGTTAC
701 AGCACCTATT  TATTGATGGG  ATCACACAAG  AAAACCCTGA  AGCCCTTCCT
40 751 AACAAATACAT  CTGGAAGACT  GACTCTATTC  CCTAGTGTTC  GTTATATCTA
801 TTCTCATTTT  ACTCCACAAA  ATCCTACAAT  ATGGCCGCAA  GTCTTTTTC
851 GACAAGGTCC  TCTAGATGAA  GATCGAGGAG  GAGGATTGA  GATCTTAGAG
901 CAATTACAAG  AGTTAGGAGT  TAGGTTTCCA  ATTTGCCCTT  CTCAGGACC
951 AGACAATCCT  AATTTTCAAG  GTTTTCAAGG  GATTCGTATC  TATTGGGAAG
45 1001 ATTCCTATCA  ACCCAATAAG  GAGGTTTAA

```

The PSORT algorithm predicts inner membrane (0.5140).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 112A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 112B) and for FACS analysis.

- 50 These experiments show that cp6430 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 113

The following *C.pneumoniae* protein (PID 4376439) was expressed <SEQ ID 225; cp6439>:

```

1  MSYDTLFLKNL EKEDSVHKIC NEIFALVPRL NTIACTEAII KNLPKADIVH
51  HLPGTITPQL AWILGVKNGF LKWSYNSWTN HRLSPKNPH KQYSNIFRNF
5  101  QDICHEKDPD LSVLQYNILN YDFNSFDRVM ATVQHRFPF GGIQNEEDLL
151  LIFNNYLQQC LDDTIVYTEV QQNIRLAHLV YPSLPEKHAR MKFYQILYRA
201  SQTFSKHGIT LRFLNCFNKT FAPQINTQEP AQRAVQWLQE VDSTFPGLFV
251  GIQSAGSESA PGACPKRLAS GYRNAYDSGF GCEAHAGEGI ETRTIFSSAK
301  VNPEGLIEIT RVTFFSLKRR QPSSLPPIRV CQLG*

```

10 The cp6439 nucleotide sequence <SEQ ID 226> is:

```

1  ATGTCTTATG ATACGTTATT CAAGAATCTT GAAAAGGAAG ATTCTGTACA
51  TAAGATATGC AATGAGATCT TTGCATTAGT ACCACGACTC AATACAATCG
101  CTTGCACCGA AGCTATCATC AAAAACCTCC CCAAAGCAGA TATCCATGTA
15  151  CACCTTCCTG GGACCATAAC ACCTCAATTA GCTTGGATTT TAGGTGTGAA
201  AAATGGGTTC TTAAATGGT CTTATAATT CTTGGACCAAT CATCGATTAC
251  TTTCTCCTAA GAATCCTCAT AAACAATACT CCAATATTTT CCGAAACTTT
301  CAAGATATCT GTCACGAAAA GGATCCGGAT TTAAGTGAT TACAATATAA
351  TATCTTAAAT TACGATTTTA ATAGCTTTGA TAGAGTGATG GCTACAGTAC
401  AAGGACATCG CTTTCCTCCT GGAGGAATCC AAAATGAAGA AGACCTTCTT
20  451  CTCATTTTCA ATAACATATC CCAGCAATGT CTGGACGATA CTATCGTGTA
501  TACTGAAGTA CAACAAAATA TCCGCCTTGC CCATGTTTTG TATCCTTCAT
551  TACCTGAAAA GCACGCGCGT ATGAAGTTT ATCAAATCTT GTATCGTGCT
601  TCGCAAAACG TTTCAAAACA CGGGATTACT TTACGATTTT TAAACTGCTT
651  CAATAAAACA TTTGCTCCAC AAATAAACAC ACAAGAACCT GCCCAAGAAG
25  701  CTGTTCATG GCTCCAAGAG GTTGATTCTA CATTTCTCTG TCTATTTGTA
751  GGGATACAAT CCGCAGGATC AGAATCTGCG CCCGAGCCT GTCTAAGCG
801  ATTAGCTTCT GGATATAGAA ATGCTTATGA CTCAGGGTTT GGTGTGAAG
851  CTCATGCTGG AGAAGGCATA GAGACCCGGA CTATTTTTC GTCAGCTAAG
901  GTAAATCCAG AGGGATGTAT CGAGATAACC CGAGTGACTT TCTCGTCTCT
30  951  TAAACGAAAA CAGCCATCTA GTTTACCCAT AAGAGTTACT TGCCAGTTAG
1001 GATAA

```

The PSORT algorithm predicts cytoplasm (0.1628).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 113A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 113B) and for FACS analysis.

These experiments show that cp6439 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 114

The following *C.pneumoniae* protein (PID 4376440) was expressed <SEQ ID 227; cp6440>:

```

40  1  LQSARRHLNT IFILDFGSQY TYVLAKQVRK LFVYCEVLPW NISVQCLKER
51  APLGIILSGG PHSVYENKAP HLDPEIYKIG IPILAICYGM QLMARDFGGT
101  VSPGVGEFGY TPIHLYPCEL FKHIVDCESL DFEIRMSHRD HVTTIPEGFN
151  VIASTSQCSI SGIENTKQRL YGLQFHPEVS DSTPTGNKIL ETFVQEICSA
45  201  PTLWNPLYIQ QDLVSKIQDT VIEVFDEVAQ SLDVQWLAQG TIYSDVIESS
251  RSGHASEVIK SHHNVGGLPK NLKLLVEPL RYLFKDEVRI LGEALGLSSY
301  LLDRHPFPGP GLTIRVIGEI LPEYLALRR ADLIFIEELR KARLYDKISQ
351  AFALFLPIKS VSVKGDERSY GYTIALRAVE STDFMTGRWA YLPCDVLSSC
401  SSRIINEIPE VSRVVDISD KPPATIEWE*

```

The cp6440 nucleotide sequence <SEQ ID 228> is:

```

50  1  TTGCAGAGTG CAAGGAGACA TTTGAACACC ATATTTATTC TAGATTTTGG
51  ATCTCAATAT ACTTATGTAT TAGCAAAGCA AGTGCAGGAG TTATTTGTAT
101  ATTGCGAAGT TCTTCCCTGG AATATCTCTG TGCAATGTTT AAAAGAAAAA
151  GCGCCTTTGG GGATCATTCT CTCAGGAGGT CCTCACTCTG TCTATGAAAA

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201 CAAGGCTCCA CATTTAGATC CTGAAATCTA TAAACTTGGC ATTCCAATTC
 251 TAGCTATTTC CTATGGCATG CAGCTTATGG CTAGAGATTT TGGAGGGACT
 301 GTAAGCCCTG GTGTAGGAGA ATTTGGATAT ACGCCCATCC ATCTGTATCC
 351 TTGTGAGCTC TTCAAACACA TCGTCGACTG CGAATCTCTA GACACAGAGA
 401 TTCGGATGAG CCATCGGGAT CATGTTACGA CAATTCCTGA AGGATTTAAT
 451 GTAATCGCAT CCACCTCACA ATGCTCGATC TCAGGAATAG AAAATACCAA
 501 ACAACGGTTG TACGGGCTGC AATTTCATCC CGAGGTTTCT GACTCCACTC
 551 CAACGGGAAA TAAGATTCTA GAACTTTTGG TTCAAGAGAT CTGTTCTGCT
 601 CCCACACTAT GGAATCCCTT GTATATTCAG CAAGACCTTG TAAGTAAAAAT
 651 TCAAGATACC GTTATTGAAG TATTTGATGA AGTCGCTCAG TCATTAGACG
 701 TACAATGGTT AGCTCAAGGA ACCATCTACT CAGATGTTAT TGAGTCCTCA
 751 CGCTCTGGAC ATGCTCCGA AGTAATAAAA TCACATCATA ATGTAGGGGG
 801 GCTTCCAAA AATCTTAAGC TGAAGTTAGT CGAGCCCTTA CGTTATTTAT
 851 TTAAAGATGA AGTTCGAATT TTAGGAGAAG CCCTAGGACT TTCTAGCTAT
 901 CTCTTGGACA GGCATCCTTT TCCTGGACCT GGCTTGACAA TTCGTGTGAT
 951 TGGAGAGATC CTTCTGAAT ATCTAGCCAT TTTACGACGG GCGGACCTCA
 1001 TCTTTATAGA AGAGCTTAGG AAAGCAAAAC TCTACGATAA AATAAGCCAA
 1051 GCCTTTGCTC TATTCTTCC TATAAAATCA GTATCTGTAA AAGGAGATTG
 1101 TAGAAGCTAT GGTATACCA TAGCATTACG TGCTGTAGAA TCTACAGATT
 1151 TCATGACAGG ACGATGGGCC TACCTTCCAT GCGATGTTCT CAGTTCTTGC
 1201 TCATCGCGAA TTATTAATGA AATACCCGAG GTAAGCCGAG TGGTCTATGA
 1251 TATTCTGAC AAGCCACCAG CAACTATAGA ATGGAATAG

The PSORT algorithm predicts cytoplasm (0.0481).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 114A) and also as
 a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used
 in a Western blot (Figure 114B) and for FACS analysis.

These experiments show that cp6440 is a surface-exposed and immunoaccessible protein, and that it
 is a useful immunogen. These properties are not evident from the sequence alone.

Example 115

The following *C.pneumoniae* protein (PID 4376475) was expressed <SEQ ID 229; cp6475>:

1 MNTYTFSPITL QKSFSLFLLR KLDSYFFFGG TRTQILVITP TNIRLAAKKR
 51 GCKVSTIEKI IKILSFILLP LVIIIFILRY FLHKFKDKQF LCIPKVISNE
 101 DEALLGSRPQ AVEKAVREIS PAFFSIPRKY QLIRIDTPKD DAPSILFPIG
 151 IEIILKDLCI DTLKQSNLFL KREMDFLGHP BEKALFDSIC SIEKDQEWMS
 201 LESKKLLITH FLKYLFSVGI BQLNPGFNPE NGRGYFSEIS TAKIHFHQHG
 251 RYGPISRSSGP IMKEI*

The cp6475 nucleotide sequence <SEQ ID 230> is:

1 ATGAATACCT ATACCTTCTC TCCTACACTT CAGAAAAGCT TCAGCCTATT
 51 TCTTTTAGAA AAATTAGACT CTTACTTTTT CTTTGAGGGG ACTCGTACAC
 101 AAATCTTAGT CATCACACCA ACCAATATTA GATTAGCAGC TAAAAAAGA
 151 GGGTGTAAGG TTTCTACTAT AGAAAAGATA ATCAAGATCC TCTCTTTTAT
 201 CCTGCTGCCC CTAGTTATCA TTGCCTTTAT ACTTCGCTAT TTCTTACATA
 251 AGAAATTCGA TAAACAGTTC TTGTGTATCC CAAAAGTCAT TTCTAACGAA
 301 GACGAAGCTC TTCTTGGATC TAGACCACAA GCAGTTGAAA AAGCAGTTCG
 351 AGAAATATCT CCAGCTTCTT TCTCTATACC AAGAAAATAC CAACTTATTA
 401 GAATCGACAC TCCTAAAGAT GACGCTCCCT CAATCCTTTT CCCTATAGGC
 451 ATAGAGATCA TTCTCAAAGA TTTATGTATT GATACACTCA AGCAATCTAA
 501 TCTTTTCTCT AAAAGAGAAA TGGATTCTCT AGGTCAATCCA GAAGAAAAAG
 551 CATTATTCTGA CTCGATATGT TCTATAGAAA AAGATCAAGA ATGGATGAGC
 601 TTGGAAAGTA AAAAAGTTT AATCAGGCAC TTCCTAAAGT ATCTCTTTGT
 651 CTCTGGAATC GAACAACTAA ATCCAGGCTT TAACCCAGAG AATGGGCGTG
 701 GGTTATTTTC AGAAATAAGT ACAGCAAAGA TCCATTTTCA TCAGCACGGT
 751 CGATATGGGC CAATCCGTTC TTCGGGACCC ATCATGAAGG AAATATAA

The PSORT algorithm predicts inner membrane (0.5373).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 115A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 115B) and for FACS analysis.

These experiments show that cp6475 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 116

The following *C.pneumoniae* protein (PID 4376482) was expressed <SEQ ID 231; cp6482>:

```

1  MLVLEALKR EFAHLKDQKP TSDQEITSLY QCLDHLEFVL LGLGQDKFLK
51  ATEDVDLFE SQKAIIDAWNA LLTKARDVLG LGDIGAIYQT IEFLGAYLSK
101 VNRRAFCLAS EIHFLLKTAIR DLNAYYLLDF RWPLCKIEEF VDWGNDCVEI
151 AKRKLCTFEK ETKEKNESLL REKHAMEKCS IQDLQRKLSL IIEIHDVSL
201 PCFSTPSQE EYQKDCLYQS RLRVLLLLLYE YTLCKTSTD FQEQARAKEE
251 FIREKPSLLE LEKGIKQTKK LEFALAKSKL ERGCLVMRKY EAAAKHSLDS
301 MFEETVTKSP RKDTE*
```

The cp6482 nucleotide sequence <SEQ ID 232> is:

```

1  ATGCTAGTAG AGTTAGAGGC TCTTAAAAGA GAGTTGCGC ATTTAAAAGA
51  CCAGAAGCCG ACAAGTGACC AAGAGATCAC TTCACTTTAT CAATGTTTGG
101 ATCATCTTGA ATTCTTTTAA CTCGGGCTGG GCCAGGACAA ATTTTAAAG
151 GCTACGGAAG ATGAAGATGT GCTTTTGTAG TCTCAAAAAG CAATCGATGC
201 GTGGAATGCT TTATTGACAA AAGCCAGAGA TGTTTTAGGT CTGGGGACA
251 TAGGTGCTAT CTATCAGACT ATAGAATTCT TGGGTGCCTA TTTATCAAAA
301 GTGAATCGGA GGGCTTTTTC TATTGCTTCG GAGATACATT TTCTAAAAAC
351 AGCAATCCGA GATTGAATG CATATTACCT GTTAGATTTT AGATGGCCTC
401 TTTGCAAGAT AGAAGAGTTT GTGGATTGGG GGAATGATTG TGTGAAATA
451 GCAAAGAGGA AGCTATGCAC TTTTGAAAAA GAAACCAAGG AGCTCAATGA
501 GAGCCTTCTT AGAGAGGAGC ATGCGATGGA GAAATGCTCG ATTCAAGATC
551 TGCAAGGAA ACTTAGCGAC ATTATTATTG AATTGCATGA TGTTCCTCTT
601 TTTTGTTTTT CTAAGACTCC CAGTCAAGAG GAGTATCAAA AGGATTGTTT
651 GTATCAATCA CGATTGAGGT ACTTATTGTT GCTGTATGAG TATACATGTT
701 TATGTAAGAC ATCCACAGAT TTCAAGAGC AGGCTAGGGC TAAAGAGGAG
751 TTCATTAGGG AGAAATTCAG CCTTCTAGAG CTCGAAAAGG GAATAAACA
801 AACTAAAGAG CTTGAGTTTG CAATTGCTAA AAGTAAGTTA GAACGGGGCT
851 GTTTAGTTAT GAGGAAGTAT GAAGCTGCCG CTAACATAG TTTAGATTCT
901 ATGTTTGAAG AAGAAACTGT GAAGTCGCCG CGGAAAGACA CAGAATAA
```

The PSORT algorithm predicts cytoplasm (0.4607).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 116A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 116B) and for FACS analysis.

These experiments show that cp6482 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 117

The following *C.pneumoniae* protein (PID 4376486) was expressed <SEQ ID 233; cp6486>:

```

1  VVVVALFILG IFFLSGSLAF LVHTSCGVLL GAALPILCIG LVLLAVALIV
51  FLCHKHKTRQ DLDYYDQDL D SLVIHKKEIP NDISELRVTF EKLQNLFPQH
101 TKDFSDLSQE LQKFLNCME KWLTLDEVT KFLIVRDRFL ETRRNFTTFG
151 EQVKGIQSN I FDLHEEKSSL YLELYRLRKD LQVLLNFFLL PPGLKLVDDYD
201 EIEAIKGLFI RLTSRLDKLD VKAQERKKFI NEMSREFKEV EKAFFDIVDRA
251 TTKKLMRAKK EPARLFMGR TESLLEMKN EALKNQGLD PENLSHPELF
301 SPYQQLILN YLNSEIVLHH YEFLISGTVT SGLTLEECEN RMRAASTGLN
```

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351 ALLVRKQLQFR GAIKSAYFEK LTRIEKELRS LQDVIKSLEL ELIHKIKDIV
401 TEET*

The cp6486 nucleotide sequence <SEQ ID 234> is:

```

5      1  GTGGTGGTTG TCGCTTTATF TATCCTTGGG ATTTCTTTT TATCTGGTTC
      51  TCTTGCATTC CTTGTTCATA CGTCTTGGCG AGTTCTTTTA GGAGCGGCGC
     101  TTCCCATACT TTGCATAGGT CTTGTTTTAT TGGCTGTAGC TCTTATTGTT
     151  TTCTTATGTC ACAAACACAA GACTCGTCAA GATTAGATT ATTATGATCA
     201  AGATTTAGAT TCTTTGGTGA TTCATAAGAA AGAGATCCCC AATGACATCT
     251  CTGAGTTGCG GGTAAACATT GAAAAGTTGC AAAATCTGTT TCAGTTCCAT
    301  ACGAAAGATT TCTCTGATCT AAGCAAAGAG CTTCAGGGTA AATTATCAA
    351  TTGCATGGAG AAATGGCTAA CTTTAGAAGA CGAAGTGACT AAATTTCTTA
    401  TTGTTCGAGA TAGATTTTTA GAAACCAGAA GAAATTTTAC CACTTTTGGA
    451  GAACAGGTTA AAGGGATCCA AAGCAATATT TTTGATTGTC ATGAGGAAAA
    501  GTCCTTCATTA TATTTAGAAT TGTATAGGCT TAGGAAAGAC CTCCAAGTTC
    551  TATTAAATTT TTTTCTGCTC CCCCAGGTA TACTCAAGGT AGATTATGAT
    601  GAAATTGAGG CTATCAAAGG TCTGTTTATA AGATTAACTT CTAGATTAGA
    651  TAAGCTTGAT GTGAAAGCTC AGGAACGTAA GAAGTTCATT AATGAAATGA
    701  GTAGGGAATT TAAAGAAGTA GAGAAAGCTT TTGATATTGT CGATAGGGCA
    751  ACAAAGAAAGC TTATGGATAG AGCCAAGAAA GAAAGTCCGG CACGTCTTTT
    801  CATGGGTAGA ACTGAGTCTC TCTTAGAAAT GAAAAAAAT GAAGAAGCCC
    851  TTAAAAATCA GGGGCTAGAT CCTGAAAATC TTTCCCATCC TGAACCTTTT
    901  AGTCCGTATC AACAGCTTTT AATTTTGAAT TATTTAAATA GCGAAATAGT
    951  TCTGCATCAT TATGAGTTCC TTATTCTGG AACAGTAAC TCTGGCCTAA
   1001  CTCTTGAAGA ATGTGAAAT CGAATGAGGG CGGCTTCTAC TGGGTTGAAC
   1051  GCCCTTCTGG TCGTAAAGCT CCAGTTCAGA GGTGCTATAA AATCTGCGTA
   1101  TTTTGAAGAA CTCACAGAGA TTGAAAAAGA GTTACGATCA CTTCAAGACG
   1151  TAATAAAGTC ATTGGAAC TAACATGATCC ATAAGATAAA AGATATAGTG
   1201  ACAGAAGAAA CTTAG

```

The PSORT algorithm predicts inner membrane (0.7474).

- 30 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 117A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 117B) and for FACS analysis.

These experiments show that cp6486 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

35 Example 118

The following *C.pneumoniae* protein (PID 4376526) was expressed <SEQ ID 235; cp6526>:

```

40      1  MSPFKKIVNR LLCYISFQKE SRTLPIIIRE PRMTTKSLGS FNSVISKNKI
      51  HFISLGC SRN LVDSEVMLGI LLKAGVESTN BIEDADYLIL NTC AFLKSAR
     101  DEAKDYLDHL IDVKKENAKI IVTGCMTSNH KDELKPWMSH IHYLLGSGDV
     151  ENILSAIBSR ESSEKISAKS YIEMGEVPRQ LSTPKHYAYL KVAEGCRKRC
     201  AFCIIPSIKG KLRSKPLDQI LKEFRILVNK SVKEIILIAQ DLGDYGDLS
     251  TDRSSQLESL LHELLKEPGD YWLRMLYLYP DEVSDGIIDL MQSNPKLLPY
     301  VDIPLQHIND RILKQMRRTT SREQILGFLE KLRKVVPQVY IRSSVIVGFP
     351  GETQEEFQEL ADFIGEGWID NLGIFLYSQE ANTPAABELPD QIPEKVRESR
     401  LKILSIIQKR NVDKHNQKLI GEKIEAVIDN YHPETNLLLT ARFYGQAPFV
     451  DPCIIVNEAK LVSHFGERCF IBITGTAGYD LVGRVVKKSQ NQALLKTSKA
     501  *

```

The cp6526 nucleotide sequence <SEQ ID 236> is:

```

50      1  ATGAGTCCCTT TTAAGAAAAAT AGTAAATCGC TTACTATGCT ATATTTCTTT
      51  TCAAAAAGAA TCAAGAACTC TCCCAATCAT TATTAGAGAA CCTAGGATGA
     101  CAACAAAAG TTTAGGATCT TTCAATTCAG TTATTTCCTAA AAATAAAATT
     151  CATTTTATTA GTTTGGGATG CTCTCGGAAC CTGTAGATA GCGAAGTCAT
     201  GCTAGGCATT CTCTTAAGG CAGGTTACGA GTCTACTAAT GAAATTGAAG
     251  ATGCTGACTA TTTAATTTTA AATACCTGTG CGTTTTTAAA AAGTGCTAGA
     301  GATGAAGCTA AAGATTATCT AGACCATCTA ATTGATGTAA AAAAAGAGAA

```

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```

351 CGCTAAAATT ATTGTAAGTG GATGCATGAC TTCCAACCAC AAAGATGAGC
401 TTAAACCCCTG GATGTCACAC ATCCATTACC TACTAGGTTT TGGGGATGTT
451 GAGAATATTC TTTCTGCTAT TGAGTCTCGT GAATCTGGAG AAAAAATCTC
501 TGCAAAGAGT TACATTGAGA TGGGAGAAGT TCCAAGACAG CTTTCCACAC
551 CAAAACACTA TGCCATTTTA AAAGTTGCTG AGGGCTGTAG AAAACGTTGT
601 GCTTTTGTGA TTATTCCTTC CATTAAAGGA AAGCTCCGCA GCAAACCTCT
651 GGATCAAATT CTTAAAGAAT TCCGCATCCT TGTAAACAAG AGTGTGAAAG
701 AGATTATATT GATAGCTCAA GACCTAGGAG ATTATGGAAA GGATCTCTCT
751 ACAGACCGCA GTTCGCAGCT AGAATCACTA TTACATGAGT TACTGAAAGA
801 CCCTGGTGAT TATTGGCTGC GGATGTGTA TTTATATCCT GATGAAGTGA
851 GTGATGGCAT TATAGATCTT ATGCAATCTA ATCCCAAAC TCTTCCCTAT
901 GTAGATATTC CCTTACAGCA CATTACGAC CGTATTTTAA AGCAAAATGCG
951 AAGAACGACT TCTAGGGAGC AAATCCTAGG ATTCCTAGAA AAATTACGTG
1001 CCAAGGTTCC TCAGGTCTAT ATCCGTTCTT CTGTTATTGT GGGTTTCCCC
1051 GGTGAAACTC AGGAAGAATT CCAGGAGTTA GCTGATTTTA TTGGTGAGGG
1101 TTGGATGAT AATCTCGGAA TTTTCTGTGA CTCTCAAGAA GCGAATACCC
1151 CGGCAGCAGA ACTCCCTGAC CAGATACCAG AAAAAGTTAA AGAATCGAGG
1201 TTGAAAATTC TATCTCAAAT TCAGAAACGC AATGTGGATA AACATAATCA
1251 GAAGCTCATT GGGGAAAAAA TAGAAGCAGT TATTGATAAC TATCATCCTG
1301 AAACGAATCT TTTACTCACT GCAAGGTTCT ATGGACAAGC TCCTGAAGTG
1351 GACCCTTGTA TTATTGTAAA TGAGGCGAAG CTGTGTTCTC ATTTTGGAGA
1401 AAGATGCTTT ATAGAAATCA CAGGGACTGC TGGTTACGAC CTTGTAGGGC
1451 GTGTTGTAAA AAAATCTCAG AACCAAGCTT TGCTAAAAAC TAGCAAAGCT
1501 TAG

```

The PSORT algorithm predicts cytoplasm (0.1296).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 118A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 118B) and for FACS analysis.

These experiments show that cp6526 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 119

The following *C.pneumoniae* protein (PID 4376528) was expressed <SEQ ID 237; cp6528>:

```

1 MKNNINNNEC YFKLDSTVDG DLLAANLKTf DTQAQGISST ETFSVQGNAT
51 FKDQVSATGL TSGTTYNLNA QNFTSSQISI DFKNNRLSNC ALPKEDCDPV
101 PANYVRSPEY PFC SKPLIGD FDFNSGESYL PLTGSEYTLY QSRNVNSIFR
151 FIGWKQSTRE LTVGGNTAIQ FLAAGTYIVS FTVGKRWGNW NGWGGAIYIN
201 NGLGQVQCES TIYSGGYAT IGTGTSIYR ASVDVAPNPN DPNASDRYRA
251 GIFYLSNGGS SAGIGNYSFS LLYYPDDR*

```

The cp6528 nucleotide sequence <SEQ ID 238> is:

```

1 ATGAAAAACA ATATTAATAA TAATGAGTGC TATTTTAAAT TAGACTCAAC
51 TGTAGATGGT GATTTGTAG CAGCCAATCT CAAGACCTTT GATACACAGG
101 CCCAAGGAAT CTCATCGACT GAAACATTTT CTGTTCAGGG GAATGCAACA
151 TTFAAGATC AAGTTTCAGC AACTGGATTA ACTTCAGGAA CTACTTATAA
201 TTFAAATGCA CAAAACTTTA CTTCCTCCCA AATCTCTATA GATTTTAAAA
251 ATAATCGTCT GAGTAATTGT GCATTGCCAA AAGAAGACTG CGATCCGGTG
301 CCAGCGAATT ATGTTCTGTT TCCCGAATAT TTTTCTGTT CCAAGCCTCT
351 GATCGGAGAT TTTGATTTTA ACTCAGGGGA ATCTTATTTG CCTCTGACTG
401 GTTCGGAATA TACTCTATAT CAGTCACGTA ATGTAAATAG TATATTTCTG
451 TTTATAGGAT GGAAGCAAAG TACACGAGAA TTAAGTGTAG GGGGAAATAC
501 TGCGATACAA TTTCTTGCA GAGGAACCTA TATCGTTTCA TTTACTGTTG
551 GTAAACGGTG GGGATGGAAT AATGGTTGGG GAGGAGCCAT TTATATCAAT
601 AATGGTTTAG GACAAGTCCA ATGTGAAAGC ACGATTTATA GTGGTGAGG
651 GTATGCAACA ATAGGTACAC TGGGGACCTC AATATATAGA GCCTCTGTAG
701 ATGTAGCTCC TAATCCTAAT GATCCGAATG CTTCGGATCG CTATAGAGCG
751 GGTATTTTCT ATCTCAGTAA CGGTGGTTCT AGTGCAGGTA TAGGGAATTA
801 CTCCTTTTCT CTCTCTATT ATCCGGACGA TAGAGGGTAG

```


The PSORT algorithm predicts cytoplasm (0.1668).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 119A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 119B) and for FACS analysis.

- 5 These experiments show that cp6528 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 120

The following *C.pneumoniae* protein (PID 4376627) was expressed <SEQ ID 239; cp6627>:

```

10      1  MKCSPLTLVP  HIFLKNDCBC  HRSCSLKIRT  IARLILGLVL  ALVSALSFVF
      51  LAAPISYAIG  GTLALAAIVI  LIITLVVALL  AKSKVLPIPN  ELQKIYNRY
     101  PKEVFFYFKT  HSLTVNELKI  FINCWKSGTD  LPPNLHKRAE  AFGIDILKSI
     151  DLTLFPEFEB  ILLQNCPLYW  LSHFIDKTES  VAGEIGLNKT  QKVYGLLGPL
     201  AFHKGYYTIF  HSYTRPLLTL  ISESQYKFLY  SKASKNQWDS  PSVKRTCEBI
     251  FKELPHNMIF  RKDVQGISQF  LFLFPHSHGIT  WEQAQMIQLI  NPDNWKMLCQ
     301  FDKAGGHCSM  ATFGGFLNTE  TNMPDPVSSN  YEPTVNFMTW  KELKVILLEKV
     351  KESPMHPASA  LVQKICVNTT  HHQNLKRWQ  FVRNTSSQWT  SSLPQYAFHA
     401  QTYKLEKKIE  SSLPIRSSL*
```

The cp6627 nucleotide sequence <SEQ ID 240> is:

```

20      1  ATGAAGTGTA  GTCCTTTAAC  ACTAGTTCCC  CATATATTTT  TAAAAAATGA
     51  CTGCGAATGT  CATAGATCTT  GTTCTTTAAA  AATTAGGACA  ATTGCCCGAC
    101  TCATTCTTGG  GCTTGTCTTA  GCTCTGTGTA  GCGCACTTTC  TTTTGTFTTC
    151  CTTGCTGCGC  CGATTAGCTA  TGCTATGGA  GGAACTTTAG  CTTTAGCCGC
    201  TATCGTAATC  TTGATTATAA  CGCTAGTCGT  AGCACTGCTA  GCTAAATCAA
    251  AGGTTCTGCC  CATCCCAAC  GAACCTCAGA  AGATTATTTA  CAATCGCTAT
    301  CCTAAAGAAG  TCTTTTATTT  CGTGAAAACA  CACTCCCTGA  CTGTTAACGA
    351  ATTAAAAATA  TTTATTAATT  GCTGAAAAG  CCGTACAGAC  CTGCCCTCGA
    401  ATTTACATAA  AAAAGCAGAG  GCTTTTCGGA  TCGATATTCT  AAAATCTATA
    451  GATTTAATCC  TGTTTCCAGA  GTTCGAAGAG  ATTCTTCTTC  AAAACTGCCC
    501  GTTATACTGG  CTCTCCCAT  TTATAGACAA  AACTGAATCT  GTTGCTGGGG
    551  AAATCGGATT  AAATAAAACA  CAAAAGTTT  ATGGTTTACT  TGGGCCCTTA
    601  GCGTTTCATA  AAGGATATAC  AACTATTTTC  CACTCTTATA  CACGCCCTCT
    651  ACTAACATTA  ATCTCAGAA  CACAGTATAA  GTTCTTATAT  AGTAAAGCGT
    701  CTAAGAATCA  ATGGGATTCT  CTTCTGTGA  AAAAAACCTG  CGAAGAAATA
    751  TTCAAGGAAC  TCCCCACAA  TATGATTTTC  CGGAAGGATG  TTCAAGGAAT
    801  CTCACAATTC  TTATTTCTTT  TCTTTTCTCA  TGGTATCACT  TGGGAACAGG
    851  CTCAGATGAT  TCAACTTATA  AATCCTGATA  ATTGGAAAT  GTTGTGTCAG
    901  TTTGATAAAG  CAGGAGGCCA  CTGTTCATG  GCAACATTIG  GAGGCTTTT
    951  GAATACTGAA  ACAAATATGT  TCGATCCAGT  ATCCTCTAAC  TATGAACCTA
   1001  CAGTGAACCT  CATGACGTGG  AAAGAATTGA  AGGTTTACT  AGAGAAAGTA
   1051  AAAGAAAGTC  CTATGCACCC  AGCGAGTGCT  CTGTTCAGA  AGATATGCGT
   1101  AAATACAACG  CACCATCAA  ATCTGTAAA  ACGATGGCAA  TTTGTTGTA
   1151  ATACGAGTTC  ACAATGGACA  TCAAGCTTAC  CTCAGTATGC  TTTCCACGCC
   1201  CAAACCTACA  AACTAGAGAA  AAAAATAGAA  AGCAGTCTCC  CTATACGATC
   1251  TTCCCTATAA
```

- 45 The PSORT algorithm predicts inner membrane (0.7198).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 120A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 120B) and for FACS analysis.

- 50 These experiments show that cp6627 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 121

The following *C.pneumoniae* protein (PID 4376629) was expressed <SEQ ID 241; cp6629>:

```

1 MSNITSPVIQ NNRSCNYYFE LKNSTTIHIV ISAILLCGAL IAFLCVAAPV
51 SYILSGALLG LGLLIALIGV ILGIKKITPM ISSKEQVFPQ ELVNRIRAHY
101 PKFVSDFVSE AKPNLKDILS FIDLLNQLHS EVGSSTNYNV SEELQOKIDT
151 FEGIARLKNE VRTASLKRLE SAASSRPLFP SLPKILQKVF PFFWLGEFIS
201 AGSKVVELHR VKKIGGSLEE DLSDIKPEM LPTYWLIPLD FRPTNSSILN
251 LHITLVLARVL TRDVFQHLKY AALNGEWNLN HSDLNMTKQQ LFAKYHAAYQ
301 SYKHLSPSL QEDEFYNLLL CIFKHRYSWK QMSLIKTVPA DLWENLCCLT
10 351 LDHTGRPDQM EFASLIGTLY TQGLIHKESE AFLSSLTLLS LDQFKTIRRO
401 STNIAFLEN LATHNSTFRS LPPI TVHPLK RSVFSQPEED ESSLLIG*
```

The cp6629 nucleotide sequence <SEQ ID 242> is:

```

1 ATGAGTAATA TAACCTCGCC AGTTATTCAA AATAATCGCT CTTGTAATTA
51 TTATTTTGAA TTAAAGAATT CAACCACTAT TCATATTGTT ATCAGTGCCA
15 101 TCTTACTCTG CGGAGCTTTG ATAGCTTTCT TGTGTGTAGC AGCTCCTGTT
151 TCCTATATTC TAAGTGGCGC ATTGTAGGA TTAGGATTAT TAATAGCCTT
201 GATTGGTGTG ATTTTAGGAA TAAAAAAAT CACGCCTATG ATTTTCATCAA
251 AAGAACAAGT ATTCCCCCAA GAACTCGTAA ATAGAATCAG GCGCCTACTAT
301 CCTAAATTTG TCTCTGATTT TGTTCAGAA GCTAAACCAA ATCTTAAAGA
20 351 TCTCATAAGT TTTATTGATC TTCTAAATCA ATTGCACTCT GAAGTGGAT
401 CATCTACAAA TTACAACGTA TCTGAAGAAC TACAACAGAA AATAGATACG
451 TTCGAGGGTA TCGCACGCTT AAAAAATGAA GTCCGCTACTG CTTCTCTTAA
501 AAGACTTGAA AGCGCTGCTT CTTCCCGTCC CCTCTTCCCC TCTTTACCAA
551 AAATCTTACA AAAGGTATTT CCAATTTTCT GGTTAGGAGA GTTTATTTCT
25 601 GCAGGCAGCA AGGTTGTAGA GCTCCATCGA GTTAAGAAAA TTGGAGGCAG
651 CCTCGAAGAA GACCTTAGTG ATTATATAAA ACCAGAGATG CTTCTTACCT
701 ATTGGTTGAT TCCTTTAGAT TTTAGACCAA CAAATTCCTC TATTCTAAAT
751 CTACACACAT TAGTTT TAGC TAGAGTCTTA ACTCGTGATG TTTTCAACA
801 TCTTAAGTAT GCAGCATTA ATGGCGAGTG GAACCTGAAT CATAGTGATC
30 851 TAAATACTAT GAAACAGCAG CTCTTTGCTA AATATCATGC GGCGTATCAA
901 TCCTATAAAC ATCTATCTCA ACCCTCTCTT CAAGAGGATG AATTCTATAA
951 CCTGCTCTTG TGTATTTT TA AGCATAGGTA CTCGTGGAAG CAGATGTCCT
1001 TAATAAAAAC AGTCCCGGCT GATTTATGGG AAAACCTCTG TTGCTTGACT
35 1051 TTAGACCATA CAGGACGACC CCAAGACATG GAATTTGCC TCTTAATTGG
1101 TACTCTCTAC ACACAAGGCC TAATTCATAA AGAAAGCGAA GCATTTCTTT
1151 CTTCAATTGAC ACTCCTTAGT TTAGATCAGT TTTAAACGAT CCGTCGTCAG
1201 TCAACCAATA TAGCGATGTT CCTTGAGAAT TTAGCAATC ATAATTCCAC
1251 CTTTAGAAGC TTACCACCTA TAACAGTCCA TCCACTCAAG AGAAGCGTCT
1301 TCTCCCAACC TGAAGAAGAC GAGTCCTCCC TGCTGATAGG TTAG
```

40 The PSORT algorithm predicts inner membrane (0.5776).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 121A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 121B) and for FACS analysis.

These experiments show that cp6629 is a surface-exposed and immunoaccessible protein, and that it
45 is a useful immunogen. These properties are not evident from the sequence alone.

Example 122

The following *C.pneumoniae* protein (PID 4376732) was expressed <SEQ ID 243; cp6732>:

```

1 MEMMSPFQQP EQCHFQVVGSL FLRPESLTRA RSDFEGRIV YEQMRVVEDA
51 AIRNLKKQT EAGLIFFFTDG EFRYSWDFD FMWGFHGVDR RRDSNDPEIG
50 101 VYLKDKISVS KHPFIEHFEP VKTFEKGNAK AKQTIPSPSQ FFHEMIFAPN
151 LKNTRKFYPT NQELIDDIVF YYRQVIQDLY AAGCRNLQLD DCAWCRLLDI
201 RAPSWYGVDS HDRLQEILEQ FLWIHNLVMK DRPEDLFVSL HVCRGDYQAE
251 FFSRRAYDSI EEPLFAKTDV DSYHYWALD DKYSGGAEPL AYVSGEKHVC
301 LGLISSNHSC IEDRDAVVSRL IYEAASYIPL ERLSLSPQCG FASCEGDHRM
```

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351 TEEBQWKIA FVKEIAKEIW G*

The cp6732 nucleotide sequence <SEQ ID 244> is:

```

1  ATGGAATGA TGAGCCCAT CCAACAACCT GAGCAATGTC ATTTTGATGT
5  51  TGTGGGAAGT TTCTTACGTC CTGAAAGTCT TACACGAGCA CGCTCTGATT
    101  TTGAAGAAGG AAGAATGTGC TATGAGCAGA TGCGAGTTGT CGAAGATGCT
    151  GCTATTCGTA ATCTCATAAA AAAGCAAACA GAAGCAGGTC TTATCTTTTT
    201  TACTGATGGG GAATCCCGTA GGTATAGTTG GGATTTGAC TTTATGTGGG
    251  GATTCCATGG CGTGGATCGT CGCAGGGACT CTAATGACCC TGAAATTGGA
    301  GTGTATCTTA AAGATAAAAT CTCCGTATCA AAACATCCGT TTATAGAACA
10  351  TTTTCGAGTTT GTCAAAACCT TTGAGAAGGG AAATGCAAAA GCAAAACAAA
    401  CGATTCCTTC TCCATCACAA TTTTCCATG AGATGATTTT TGCTCCTAAT
    451  CTGAAAAATA CTCGGAAGTT TTATCCTACG AATCAAGAGC TAATTGATGA
    501  TATTGTCTTT TATTATCGCC AAGTCATCCA AGATCTTTAT GCTGCAGGTT
    551  GTCGTAATTT GCAGTTGGAC GATTGTGCTT GGTGTCGCCT CTTGGATATA
15  601  CGAGCGCCTT CTTGGTATGG TGTGATCTT CATGACAGGT TGCAGGAAAT
    651  TTTAGAACAG TTTTATATGGA TCCATAATTT AGTGATGAAG GATAGACCCG
    701  AGGATCTTTT TGTAACTCTG CATGCTCTGTC GTGGTGATTA TCAGGCCGAG
    751  TTTTCTCTTA GACGAGCTTA TGATTCTATA GAGGAGCCTT TATTTGCTAA
    801  GACCGATGTG GATAGTTATC ACTATTATTG GGCTCTTGAT GATAAGTATT
20  851  CAGGAGGTGC TGAGCCTTTA GCTTACGTCT CTGGAGAGAA ACACGCTCTGC
    901  TTGGGATTGA TCTCCAGCAA CCATTCCTGT ATTGAAGATC GAGATGCTGT
    951  GGTTTCTCGT ATTTATGAAG CTGCGAGCTA CATTCCTTTA GAGAGACTTT
    1001  CTTTGAAGCC GCAATGTGGG TTTGCTTCTT GTGAGGGAGA CCATAGAATG
    1051  ACTGAAGAAG AACAGTGGAA GAAGATCGCC TTTGTGAAAG AGATTGCTAA
25  1101  AGAGATCTGG GGATAA

```

The PSORT algorithm predicts cytoplasm (0.2196).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 122A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 122B) and for FACS analysis.

- 30 These experiments show that cp6732 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 123

The following *C.pneumoniae* protein (PID 4376738) was expressed <SEQ ID 245; cp6738>:

```

35 1  VWLRFLLVLS YDEKEDVVV VCNHSEPNIL GLPPEAVSQL IEELSDEGYS
    51  YLNVVRCDLS GETTVQQRLL LNADEGRSMT VVISELPEGH PDIRNLQLAS
    101  ERIFVSREKE AADAYASGCK VVAFDDEHLP WVSSHAYAE EIREKQBQTM
    151  QGSLTEEQLG ALLCNFVSTE KNLAFALDAV IKQSVWRFRN PDLFAYEREA
    201  LEASVTDALV SYVSNLDMIP YTSSQGIVIE DSSIVRTSQE HTLIVNCAAF
    251  DKLASQIEFL CPDVLPIISG KDPLISDDRD EELNPKVSSA ADSKDKT*

```

40 The cp6738 nucleotide sequence <SEQ ID 246> is:

```

1  GTGTGGCTGC GCTTTTACT TTTAGTGTC TATGATGAGA AGGAGAAAGA
51  CGTAGTTGTC GTTTGTAATC ATTCTGAACC TAATATCCTC GGCCTGCCTC
101  CTGAAGCAGT CTCTCAGCTT ATTGAAGAGC TTAGCGATGA AGGCTATAGC
151  TATCTGAATG TAGTCCGTTG TGATCTCTCC GGGGAGACTA CGGTTCAACA
45  201  ACGTCTGCTA TTGAATGCCG ATGAAGGGAG ATCTATGACG GTGGTGATCT
    251  CAGAGCTTCC TGAAGGGCAC CCCGATATTC GGAATTGCA GTTGGCATCC
    301  GAAAGAATTT TTGTTTCTCG TGAAAAGAGAA GCTGCTGATG CCTATGCTTC
    351  AGGATGTAAA GTGGTCGCTT TCGATGATGA GCATCTCCCT TGGGTCTCCA
    401  GTCATATTGC CTACGCGGAG GAGATCAGAG AGAAACAAGA ACAAACAATG
50  451  CAAGGGTCTT TAACGAAGA GCAGTTAGGA GCACTCCTCT GCAACACAGT
    501  CTCCACAGAG AAAAATCTAG CCTTTGCTCT AGACGCCGTG ATAAAACAGT
    551  CTGTGTGGAG ATTCCGCAAT CCGGATCTTT TTGCTTATGA GAGAGAAGCT
    601  CTAGAGGCTT CAGTAACAGA TGCTTTAGTA TCTTACGTTT CAAATTAGA
    651  CATGATACCG TACACAAGTT CTCAGGGCAT AGTCATAGAA GATAGTAGTA
55  701  TCGTCCGTAC CTCTCAAGAG CATACACTCA TTGTGAACTG TGCAGCATTC

```

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```

751 GATAAGTTAG CGAGCCAAAT AGAGTTCTTA TGCCCCAGTG ACGTGTGCGC
801 CATTTCTGGT AAAGACCCTT TGATTCTCTGA TGATGAGGAT GAGGAAGTGA
851 ATCCTAAAGT TTCATCTGCT GCAGACTCTA AAGATAAAAC CTAG

```

The PSORT algorithm predicts cytoplasm (0.1587).

- 5 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 123A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 123B) and for FACS analysis.

These experiments show that cp6738 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

10 Example 124

The following *C.pneumoniae* protein (PID 4376739) was expressed <SEQ ID 247; cp6739>:

```

1 MTHCLHGWFV VVRHHFVQAF NFSRPLYSRI THFALGVIKA IPIVGHVLMG
51 VDWLLSHCFE RGVSHPGFPS DIAPILKVEK IAGRDHISRI ENQLKSLRKT
101 IEVEDLDKVH GQYQENPYAD MASSEVLKLD KGVHVSELGK AFSRVRNRIT
15 151 RSYSYAPTPO LDSIAIVGID LVSPBQENL VRLANEVIQL YPKSKTTLYL
201 LIDFNKEWVG DISSDKKKQL RSLGLHSEVQ CLSVLEPQGA EGEDTKHFDL
251 MVGCGYKDSY LREGKILQQA LGTSLGTVPW VNMHTLPSR YRSRLSLPIN
301 TEKDKTELYK EISRTHQLH TLGMGLGAQD SGLLLDRQRL HAPLSQGS HC
351 HSYLADLTBE ELKILLFSAF VDAKNISKKE LREVSILNFAN DTSVECGCAF
20 401 YF*

```

The cp6739 nucleotide sequence <SEQ ID 248> is:

```

1 ATGACTCATT GCTTACATGG TTGGTTTCTT GTAGTTCGTC ATCATTCTGT
51 GCAGGCGTTT AATTCTCTAC GTCCTTTATA TTCTCGAATT ACCCACTTCG
25 101 CTTTAGGGGT GATTAGGCC ATCCCCATTG TAGGGCATCT TGTTATGGGA
151 GTCGATTGGT TGATCTCTCA TTGCTTCGAG AGGGGAGTCT CACACCCTGG
201 GTTCCCTTCA GATATGCTC CTATACTGAA AGTAGAAAAG ATCGCGGGCC
251 GAGATCATAT TTCTAGAATC GAAAATCAGC TAAAGAGCCT TAGGAAAAC T
301 ATCGAGGTTG AAGATCTAGA TAAAGTCCAC GGGCAATATC AAGAGAATCC
351 TTATGCAGAT ATGGCCTCTA GTGAGGTCTT TAAACTCGAT AAGGGAGTTC
30 401 ATGTAGTCGA GCTTGGCAAA GCCTTTTCTA GAGTTCGCAA TCGCATCACC
451 AGATCTTATA GTTATGCCCC TACTCCTCAG TTGGACTCTA TAGCTATTGT
501 TGGTATAGAT CTCGTCAGTC CTGAAGAACA AGAGAATTTA GTACGCTTGG
551 CGAATGAGGT CATTCAACTC TATCCCAAAT CAAAGACAAC TCTATATCTT
601 CTTATCGATT TTAATAAGGA GTGGGTAGGG GATATCTCCT CTGATAAGGA
35 651 AAAACAGCTC CGTTCTCTAG GTCTACATTC TGAAGTTCAG TGTCPTTCCG
701 TCTTGGAAAC TCAGGGTGCC GAGGGCGAAG ATACGAAACA CTTTGACCTT
751 ATGGTTCGGT GTTATGGGAA GGATTCTTAC TTAAGGGAGG GTAAAAATTT
801 ACAGCAGGCC CTAGGGAATT CGTTAGGTAC TGTTCCTCTG GTGAATGTTA
851 TGCACACATT GCCATCTAGG TATAGATCTC GGCTTTCTCT ACCATATAAAT
40 901 ACCGAAAAGG ATAAGACAGA GCTTTATAAA GAGATTCTCT GTACACACCA
951 TCAGTTGCAT ACTTTGGGAA TGGGACTTGG AGCCCAGGAT TCAGGATTGC
1001 TCTTAGACCG GCAACGACTC CATGCTCCTT TATCTCAAGG GTCTCACTGC
1051 CATTCTTATC TTGCAGATCT CACCCATGAA GAGCTGAAA TTTTGTATT
1101 TTCAGCATT GTGGATGCTA AGAACATAAG TAAGAAAGAG CTTCTGTGAGG
45 1151 TATCTCTAAA TTTTGCTAAC GATACTTCCG TAGAGTGTGG CTGCGCTTTT
1201 TACTTTTAG

```

The PSORT algorithm predicts inner membrane (0.2190).

- 50 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 124A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 124B) and for FACS analysis.

-150-

These experiments show that cp6739 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 125

The following *C.pneumoniae* protein (PID 4376741) was expressed <SEQ ID 249; cp6741>:

```

5      1  MASCLSAWFS  IVREHFYRAF  DFSLPFCARI  TEFVLGVIKG  IPVVGHIIVG
      51  IEWLVSRYLE  SFVTKPTFVS  DVVSLKTEK  VAGRDHIARV  VETLKRQ RVA
     101  VAPEDEDKVH  GKIPVHPFGG  IQPVEVLTL  Y  PEVQDATLGL  AFSKIRNRVR
     151  QAYLQAPRPK  LQKIYIIGND  MNPFEVDDFL  HLAARLCNETQ  RLYPDATISL
     201  YLTASGGRNA  MDKKNRKLSS  DCELNPKIAC  LDFNQGDVVK  QATCDCWMVY
10    251  HGENDQGTLN  QIQEELKSG  EETPWIHVGQ  KPLSQSLWDF  SPFSSLEMKG
     301  DKEKALEYSE  LEKEQLYSRL  VYVGERSSVL  SLGFGDSRSG  ILMDPKRVHA
     351  PLSEGHYCHS  YLADLENPGL  QKTILAAFLN  PKELSSTILQ  PISLNLILNS
     401  KTYLRQHFGF  FERMSRSDRN  VVVVVCDSSW  GTDWKEEPSF  QHFIMELECR
     451  GYSHFNIFAF  RSNSMCVEER  RILNESSQEK  AFTMIFCEDS  VSQGDIRCLH
15    501  LASEGMLCGK  ECVAVDVYTS  GCANFMMEEV  LTLERESNLW  NRKHGLWKRE
     551  VRKQRQEAAL  DQDSEIYVC  NQLTAQQNFA  CS*

```

The cp6741 nucleotide sequence <SEQ ID 250> is:

```

      1  ATGGCTTCTT  GTTTATCTGC  CTGGTTTTCT  ATAGTTCGTG  AGCACTTTTA
     51  TCGAGCCTTT  GATTTTCTCT  TGCCGTTTGT  TGCTCGTATT  ACGGAATTTG
20    101  TATTAGGGGT  CATCAAGGGG  ATCCCTGTTG  TGGGTCACAT  TATTGTTGGG
     151  ATAGAGTGGC  TCGTTTCTAG  GTATTTAGAG  AGTTTCGTGA  CCAAGCCGAC
     201  ATTTCTCTCT  GATGTGGTGA  GTCTTCTGAA  AACAGAGAAA  GTTGCTGGTC
     251  GCGATCACAT  TGCTCGTGTA  GTGGAGACTT  TGAAGAGGCA  GAGAGTCGCT
30    301  TGGGCTCCTG  AAGATGAGGA  TAAGGTCCAT  GGGAGAGATT  CTGTGCATCC
     351  TTTCCGGGGA  ATCCAACCTG  TAGAAGTTCT  CACTCTCTAT  CCCGAAGTTC
     401  AAGATGCAAC  GTTAGGGCTT  GCCTTCTCTA  AAATTCGTAA  TCGTGTAAGA
     451  CAGGCGTATT  TGCAAGCTCC  ACGGCCAAAA  CTGCAGAAGA  TTTACATCAT
50    501  AGGAAACGAT  ATGAATCCTT  TTGAAGTTGA  CGACTTCTTG  CATCTAGCCC
     551  GTCTCTGTAA  TGAAACTCAA  AGACTCTATC  CTGACGCTAC  GATTTCTCTA
30    601  TATCTAACAG  CTTCTGGTGG  TCGCAATGCT  ATGGCAAAAA  AGAATCGGAA
     651  GTTACTTAGT  GATTGCGAAC  TAAACCCCAA  GATTGCTTGT  TTGACATTTA
70    701  ATCAGGGTGA  TGTAATCAAA  CAAGCAACTT  GTGACTGTGT  GATGGTGTAT
     751  CATGGGGAGA  ATGATCAAGG  TACGTTGAAT  CAGATTCAGG  AAGAGTTAGA
80    801  AAAGTCAGGG  GAGGAAACCC  CTTGGATTCA  TGTGGGGCAA  AAGCCTCTTT
     851  CACAATCCTT  GTGGGATTTT  TCTCCATTTT  CATCTTTGGA  GATGAAGGGA
35    901  GATAAAGAGA  AAGCTCTAGA  GACTCTGAA  TTAGAAAAAG  AACAGCTATA
     951  TTCTCGATTG  GTATACGTAG  GAGAGCGCTC  TTCCGTTCTT  AGTTTGGGGT
100   1001  TTGGAGATAG  TCGGTCAGGG  ATCTTGATGG  ACCCAAAACG  GGTGCATGCT
105   1051  CCCTTATCTG  AAGGGCATT  TTGTCAATCC  TACCTTCAG  ACTTAGAAAA
40    1101  TCCCGGGTTA  CAAAAACAA  TTTAGCGGC  ATTTCTGAAT  CCTAAGGAGT
     1151  TGAGCAGTAC  CATACTGCAA  CCTATATCTC  TAAATCTTAT  CTTAAATAGC
     1201  AAAACTTACT  TAAGGCAGCA  CTTTGGCTTT  TTTGAGAGGA  TGAGCAGAAG
125   1251  TGATCGCAAT  GTGGTTGTCT  TTGTATGTGA  TTCTTGGTGG  GGTACCGACT
130   1301  GGAAGGAGGA  GCCAAGCTTC  CAACACTTTA  TTATGGAGCT  AGAGTGTCTG
45    1351  GGGTATTCGC  ACTTCAATAT  TTTTGCCTTT  AGATCTAATA  GCATGTGTGT
     1401  AGAAGAACGT  AGGATCTTAA  ATGAAAGTTC  TCAAGAGAAA  GCCTTTACCA
     1451  TGATTTTCTG  TGAGGATTCA  GTATCTCAAG  GAGATATCCG  CTGTTTGCAT
150   1501  TTGGCGTCTG  AAGGAATGCT  TTGTGGTAAA  GAGTGTATG  CTGTGATGT
155   1551  CTATACGTCA  GGATGCGCGA  ACTTTATGAT  GGAAGAAGTC  TTAACTTTGG
50    1601  AGCGAGAATC  TAATCTGTGG  AATAGAAAGC  ATGGTCTTTG  GAAAAGAGAA
     1651  GTTAGAAAAC  AGAAACAAGA  AGCTGCTTTG  GATCAAGACG  AGAGCGAGAT
170   1701  TTACGTTTGT  AATCAGCTGA  CGGCGCAACA  GAACCTCGCT  TGTTCCTGA

```

The PSORT algorithm predicts inner membrane (0.2869).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 125A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 125B) and for FACS analysis.

These experiments show that cp6741 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 126

The following *C.pneumoniae* protein (PID 4376742) was expressed <SEQ ID 251; cp6742>:

```

5      1  LFVSNFIFFV  VMPIPYISSW  ISTVRQHFVK  AFDFSRRPFC  RVTNFGALGVI
      51  KAIPVGHIV  MGMEWLVS  VAGIITRSS  TSDVVQIVKT  EKALGRDHIS
     101  RVAEILQRER  GTITPENQDK  VHGFPPVCP  GRLKSEETLK  LKPGEREGTL
     151  DTVFSPINTR  VTRAYLQAPR  PEIRTISIV  SKLKTPODFS  QFVSLANETQ
     201  RLHPEALVCL  YLTGLNRESQ  MCDTTTAEKK  QYLHNSGLDS  RIQCKDSKED
10    251  DAGSPENPEL  WIGYYSREQ  HNIDGQYIQ  CLGKSADPIP  WIHVTEDTKD
     301  FYYPPNFTSY  SHTRQSTDPT  SPPRLPESEG  DKDSLYGQLS  RSYHHEYMLG
     351  LGLKPEDAGL  LMDPDRIYAP  LSQGHYCHSY  LADIENEDLR  TLVLSPLDLP
     401  GNLSSSEDLR  VAFNIARLPL  ELDSLFFRLV  AGQQEGRNIV  TLAHGTPRPE
     451  DLDPDSMNIL  TRRLQMSGYS  YLNIFYSKSR  KMIVKERQFF  GDRSEKGSFT
15    501  LILFEDPISA  ADFRCLQLAA  EGMVAKDLPS  VADICASGCS  CIQFSEMOSP
     551  QAIEYRQWEA  RVEDEAGEEA  REPVIYSQDQ  LSSMLTTQQN  FVFSLDAVVK
     601  QAIWRFRSKG  LLTMRKALG  EEFLTAIFY  LGSQERNENM  GKRTTEEHEV
     651  VISFEELDRM  VQVLPAEVPA  DSGNDPTRPV  PNPDSNPDS  QNEGS*

```

The cp6742 nucleotide sequence <SEQ ID 252> is:

```

20      1  TTGTTTGT  CTAATTTTAT  TTTTFTTGT  GTTATGCCAA  TTCCCTATAT
      51  TTCTTCTT  ATTCTTACCG  TTCGACAGCA  TTTTGTAAAG  GCGTTTGATT
     101  TCTCTCGT  CTTTCTGTCT  AGGGTTACGA  ATTTTGCTTT  AGGGGTCATC
     151  AAGGCCAT  CTATTGTAGG  ACATATTGTC  ATGGGGATGG  AGTGGTGTAG
     201  TTCTTCCT  GTTGCCGGGA  TTATTACTAG  GTCCTCCTTT  ACCTCAGATG
25    251  TCGTTCAG  TGTAAAGACT  GAGAAGGCGT  TAGGTCGAGA  TCATATATCT
     301  CGAGTGGC  AGATATTGCA  AAGAGAAAGG  GGGACCATAA  CTCCTGAGAA
     351  TCAAGATA  GTGCATGGGA  AGTTCTCTGT  CTGTCCTTTT  GGTCTGTTAA
     401  AATCCGAG  AACTTTAAAA  CTTAAGCCGG  GAGAAAGAGA  GGGAACTTTA
     451  GATACTGT  TTTCTCCGAT  TCGCACGCGC  GTGACTCGTG  CGTACTTACA
30    501  GGCCCCCG  CCCGAAATAC  GTACGATTTC  TATTGTGGGT  TCGAACTTTA
     551  AAACCTCT  AGATTTCTCG  CAATTTGTGA  GTCTCGCGAA  TGAAACGCAG
     601  AGACTGCA  CTGAAGCGTT  AGTTTGTCTG  TATTGACAG  GCTTGAATCG
     651  CGAATCTC  ATGTGCGATA  CAACTACTGC  AGAGAAGAAG  CAGTACCTAC
     701  ATAACCTC  TCTCGACTCT  AGAATCCAGT  GCAAAGACAG  TAAAGAAGAC
35    751  GACGCTGG  CTCTGAAAA  TCCCGAACTT  TGGATTGGCT  ATTATTCACG
     801  AGAGCAAC  CATAATATAG  ACGGGCAGTA  TATTTCAGCA  TGTCTAGGGA
     851  AGAGTCAG  TCCAATTCCT  TGGATTTCAT  TTAAGTGAAG  CACAAAGGAT
     901  TTTTATTA  CACCAAACTT  TACTTCATAC  TCACATACAA  GACAATCTAC
     951  AGACCCA  TCGCCACCAA  GACTCCCTGA  AAGTGAGGGG  GATAAGGATT
40   1001  CCTTGTAC  ACAACTGAGT  CGATCGTATC  ACCATGAGTA  TATGCTTGGT
     1051  TTGGGATA  AACCAGAGGA  TGCAGGACTC  CTGATGGACC  CGGATAGAAT
     1101  CTATGCTC  CTATCCCAAG  GGCATTATTG  TCATTCTTAC  CTTGCGGATA
     1151  TAGAAAAT  GGATCTACGA  ACTTTAGTCC  TTTTCGCTTT  CCTAGATCCT
     1201  GGCAATCT  GTAGCGAGGA  TCTTCGTCTT  GTAGCATTCA  ATATCGCTAG
45   1251  ATTGCCAT  GAATTGGACT  CGTTATTTT  CCGCCTTGT  GCGGGTCAGC
     1301  AAGAAGGG  AAACATAGTT  ACCCTTGCCC  ACGGAACTCC  TCGTCCAGAA
     1351  GATCTTGA  CTGACTCAAT  GAACATCTTG  ACCAGAAGAT  TACAAATGTC
     1401  TGGATATA  TATTTGAACA  TTTTCTCCTA  TAAATCACGG  AAAATGATTG
     1451  TAAAAGA  TCAGTTCTTT  GGAGATCGTT  CTGAAGGGAA  GTCTTTCACA
50   1501  TTGATCTT  TTGAGGATCC  CATTAGTGCA  GCAGATTTCC  GTTGTTTGCA
     1551  GCTAGCTG  GAAGGTATGG  TTGCTAAGGA  TCTCCCAGC  GTAGCAGATA
     1601  TTTGTGCC  TGGATGTTCC  TGCATTCAGT  TTTCTGAGAT  GCAGAGTCCT
     1651  CAGGCTATT  AATATAGACA  ATGGGAGGCA  CGTGTCAAG  ATGAAGCAGG
     1701  AGAAGAAG  AGAGAAACCA  TAATTTATTC  TCAGGATCAA  TTGAGCAGCA
55   1751  TGCTCACT  ACAACAGAA  TTTGTATTTT  CTCTAGATGC  TGTGGTAAAA
     1801  CAGGCGAT  GGAGATTCCG  TTCGAAAGGT  CTTCTTACTA  TGGAAAGAAA
     1851  GGCAC TAGG  GAGGAGTTCT  TAACTGCGAT  ATTTTCTTAT  TTAGGGAGTC
     1901  AGGACGTA  TGGAATATG  GGGAAAAGAA  CTACCGAAGA  ACATGAGGTC
     1951  GTTATCAG  TCGAAGAGCT  AGATCGCATG  GTGCAAGTCC  TCCAGCCGA
60   2001  AGTCCCTG  GATTCAAGCA  ATGATCCTAC  GCGTCCCGTT  CCTAATCCAG
     2051  ATAGTAACC  TGATTCTCTG  CAAAATGAAG  GCAGTTAG

```

The PSORT algorithm predicts inner membrane (0.2338).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 126A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 126B) and for FACS analysis.

- 5 These experiments show that cp6742 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 127

The following *C.pneumoniae* protein (PID 4376744) was expressed <SEQ ID 253; cp6744>:

```

10      1  VIQHLNLFAL  EETPSISVQY  QEQEKLSPCD  HSPBIGKKKR  WNKLESFSTY
      51  CSLFMSVKDH  YKLNLGIONS  LSGWLLDPYR  VCAPLSSPYS  CPSYLLDLQN
     101  KELRRSLLST  FLDPKNLTSE  TFRSVSINFG  NSSFGQRWSE  FLSRVLHDEK
     151  EKHVAVVCND  AKLLEEGLSP  EALSILLEEDL  RESGYSYLN  LSVSPEGVSK
     201  VQERQILRRD  LQGRSFTVMI  TDLPLGSEDI  RSLQLASDRI  LVSSSLDAAD
     251  ACASGCKVLV  YENPNASWAQ  ELENFYKQVE  RRR*

```

- 15 The cp6744 nucleotide sequence <SEQ ID 254> is:

```

      1  GTGATACAAC  ATCTTCTAAA  CTTTGCTCTA  GAAGAGACCC  CTTCCATTTC
     51  CGTGCAATAC  CAAGAACAAG  AGAAGCTCTC  TCCGTGCGAT  CATTTCCCAG
    101  AAATAGGTAA  AAAGAAAAGA  TGAATAAGC  TGGAAATCCTT  CTCCACGTAT
    151  TGTTCCTCTGT  TTAGTCTCTGT  TAAGGATCAT  TATAAGCTGA  ATCTAGGAAT
    201  TCAGAATTCC  CTGTCAGGGT  GGCTTCTGGA  TCCCTATAGG  GTTTGCGCGC
    251  CTTTATCTTC  ACCGTACTCG  TGTCTTCTCT  ATCTTTTAGA  TTTGCAAAAC
    301  AAAGAGCTAC  GTCGTTCCCT  TCTGTCAACG  TTTCTAGACC  CTA AAAATCT
    351  CACTAGCGAA  ACATTCGGTT  CTGTCTCTAT  AAACCTTGGC  AACTCTTCGT
    401  TTGGACAGAG  ATGGTCAGAG  TTTCTATCTC  GTGTTCTGCA  CGACGAGAAA
    451  GAAAAGCAGC  TAGCTGTGTG  TTGTAATGAT  GCAAAACTTC  TGGAAGAAGG
    501  ATTGTCCCCA  GAGGCATTGT  CTCTATTAGA  AGAAGACTTA  AGAGAATCAG
    551  GGTATTCGTA  TCTAAACATT  CTCTCGGTGA  GCCCCGAAGG  AGTCTCCAAG
    601  GTTCAGGAAC  GTCAGATTCT  AAGGCGAGAT  CTCCAAGGAC  GGTCTTTTAC
    651  TGTCATGATT  ACAGATCTTC  CTTTAGGTAG  CGAAGATATC  CGTAGTTTAC
    701  AATTAGCCTC  GGATAGGATT  TTAGTCTCCA  GTTCTCTTGA  TGCCGCGGAT
    751  GCATGTGCTT  CGGGATGTAA  AGTCTTAGTC  TACGAAAATC  CAAATGCATC
    801  CTGGGCTCAG  GAATTGGAGA  ACTTCTACAA  ACAAGTTGAG  AGAAGAAGGT
    851  AG

```

The PSORT algorithm predicts cytoplasm (0.3833).

- 35 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 127A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 127B) and for FACS analysis.

These experiments show that cp6744 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

40 Example 128

The following *C.pneumoniae* protein (PID 4376745) was expressed <SEQ ID 255; cp6745>:

```

      1  VACPSSSWF  TVVRQHFNVA  FDFTHPVCSR  ITNFALGIK  AIPVLGHIVM
     51  GIEWLISWIP  RHTVRHGMFT  SDVSSAIKVE  QTRGHNC LAP  LEAYLSSLRV
    101  PISQEDLGKV  HGRTPEDPFV  DITPTEIVQL  LPDEELSTVD  EALQGVRSRL
    151  TYAYRSVEKP  MIQDLALVGF  GLRDSADLIN  FVRLANGVQN  HYPHTKVKLY
    201  LAKNLADVWD  CEISEEEKGQ  LRALGLDPKI  ESISLTSAGL  PSVPEVATVD
    251  FMITCYGKDQ  EVQDP*

```

The cp6745 nucleotide sequence <SEQ ID 256> is:

```

1  GTGGCTTGTC CAAGTATTTC TTCTTGGTTT ACTGTCGTTT GACAGCATT
51  TGTAACGCC TTGATTTTCA CCCATCCCGT TTGTTCTCGG ATTACAAAT
101  TTGCTTTGGG GATCATTAAG GCAATCCCG TATTAGGACA CATTGTCATG
151  GGAATCGAGT GGTGATTTTC CTGGATTCCC AGACACACCG TTCGTCATGG
201  AATGTTTACT TCTGATGCTT CTAGTGCTAT TAAAGTAGAA CAAACACGGG
251  GTCATAATTG TTTAGCTCCC CTAGAAGCCT ATTTAAGTAG CTGAGAGTC
301  CCCATTTCCT AAGAAGATCT AGGCAAAGTA CACGGGAGAA CCCCAGAAGA
351  TCCCTTCGTA GATATCACAC CCACAGAAAT TGTCCAACCT CTCCTGATG
401  AAGAACTCTC TACTGTAGAT GAGGCACTGC AAGGCGTTTC TAGTAGGTTA
451  ACCTATGCCT ATAGGTCCGT AGAGAAACCT ATGATTCAGG ATCTTGCTCT
501  TGTGGGTTTT GGTCTCCGAG ATTCTGCGGA CCTCATAAAT TTCGTGCGTC
551  TTGCTAATGG CGTGCAGAA CACTATCCCC ATACTAAAGT GAAGCTCTAT
601  TTAGCGAAGA ACTTGCAGTA TGTCTGGGAC TGTGAAATTT CTGAAGAGGA
651  AAAAGGGCAA CTCCGAGCTC TAGGTTTAGA CCCTAAAATA GAGAGTATAT
701  CCCTTACGAG TGCAGGTCTT CCTTCAGTGC CAGAAAGTCG TACTGTCGAT
751  TTTATGATTA CCTGTACGG GAAAGATCAG GAAGTCCAAG ATCCCTAG

```

The PSORT algorithm predicts inner membrane (0.2253).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 128A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 128B) and for FACS analysis.

These experiments show that cp6745 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 129

The following *C.pneumoniae* protein (PID 4376747) was expressed <SEQ ID 257; cp6747>:

```

1  MMKQGVGQDA KELYTFLSRG NEHYQPCLWF SLKEELGFLF DEKMLCAPLS
51  EDHYCHSYLV DLVDQHLKDL ILSMFLDPQN ISAGELLKVS INVGDSPSPL
101  QQKDFLSMVL RDETGNVNVV VFKGVLSPAL TQVCKLVEEL NSKDYSYLN
151  PSCHGDSPPQ LLFRKELEGT SGRYFTVICA LYLGDFTDMRS LQLASERIMV
201  SREFDLVDAY AARCKLLKID HTNWRPGTFS RHADFADAVID VSAGFNSREF
251  KLITQANQGI LESGELPLPS KTFWEGFLAF CDRVTVTRHF IPMLDAATKQ
301  AVWTHKHPSL IDKECEALDL KTQCLPSIVS YLEYVTNSHE KTSKGPFIQK
351  EIIADCSPLK EALFPGSDED VPSTSEDPSP DHPSDLBDS*

```

The cp6747 nucleotide sequence <SEQ ID 258> is:

```

35  1  ATGATGAAAC AAGGAGTCGG GCAGGATGCT AAAGAGCTAT ACACATTTCT
51  ATCTCGTGGG AATGAGCATT ACCAACCCTG TCTATGGTTC AGTCTCGAAG
101  AGGAACTCGG ATTCTTTTTC GATGAAAAA TGCTCTGCGC CCCTCTATCT
151  GAGGATCACT ATTGCCACTC GTATCTTGTA GATCTAGTGG ATCAACATTT
201  AAAGGATTTA ATATTATCGA TGTTTTTAGA TCCTCAGAAAT ATCTCAGCAG
40  251  GAGAACTCCT CAAGGTCTCT ATAAACGTTG GAGATTCTTT TTCTCCTCTA
301  CAACAGAAAG ATTTCTCTCT GATGGTCTTA CGTGATGAAA CGGGAAAAAA
351  CGTCTGCTGT GTTTTTAAAG GAGTCTCTCT CTTACCCGCA ACCCAAGTCT
401  GCAAATTAGT AGAGGAATTG AACTCTAAGG ACTACTCCTA CCTCAATATA
45  451  TTTTCTTGTC ACGGAGATAG TAGTCCTCAG CTTTTATTCC GTAAGGAATT
501  AGAGGGAACT TCAGGCGGTT ATTTTACAGT GATTTGCGCT TTATATCTAG
551  GGGATACAGA CATGCGTAGT TTACAACCTG CTTCTGAAAG GATCATGGTC
601  TCTAGAGAGT TTGATCTTGT AGATGCCTAT GCTGCAAGAT GCAAGCTCTT
651  GAAAATCGAT CATACAAATT GGAGACCTGG AACTTTCAGT CGCCACGCCG
701  ATTTTCGAGA TGCTGTAGAC GTATCAGCAG GATTTAACTC AAGAGAATTT
50  751  AAAGTATTTA CGCAGCGCAA TCAAGGGATC CTAGAGTCTG GAGAACTCCC
801  GCTCCCTTCA AAAACCTTCT GGAAGGATT CTTAGCATTC TGTGATCGAG
851  TGACTGTACAC GAGACACTTC ATTCCAATGT TAGACGCCG CATAAAGCAA
901  GCGGTATGGA CTCATAAACA TCCCAGCTTG ATAGATAAAG AGTGTGAAGC
951  CCTAGACTTG AAAACACAGT GCTTGCCATC TATCGTATCG TACCTTGAAT
55  1001  ATGTCACAAA CTCTCACGAA AAAACATCGA AAGGCCCGTT CATACAAAAA
1051  GAGATTATCG CAGACTGTTC TCCTCTTAAA GAGGCGCTCT TCCCAGGTTT

```


-154-

1101 TGATGAAGAT GTTCCCTCTA CCTCTGAGGA TCCTTCAGAT GATCATCCTT
1151 CGGATCTTGA AGACTCTTAA

The PSORT algorithm predicts inner membrane (0.1447).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 129A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 129B) and for FACS analysis.

These experiments show that cp6747 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 130

The following *C.pneumoniae* protein (PID 4376756) was expressed <SEQ ID 259; cp6756>:

1 MASGIGGSSG LGKIPPKDNG DRSRSPSPKG ELGSHEISLP PQEHGEEGAS
51 GSSHIHSSSS FLPEDQESQS SSSAASSPGF FSRVRSGVDR ALKSFGNFFS
101 ABSTSQARET RQAFVRLSKT ITADERRDVD SSSAAATEAR VAEDASVSGE
151 NPSQGVPEFS SGPEPQRLFS LPSVKKQSG LRLVQTVRDR IVLPSGAPPT
201 DSEPLSLYEL NLRSLSLRQE LSDIQSNDQL TPEEKAEATV TIQQLIQITE
251 FQCGYMEATQ SSVSLAEARF KGVETSDEIN SLCSSELTDPE LQELMSDGDS
301 LQNLLEDATAD DLEAALSHTR LSFSLDDNPT PIDNNPTLIS QKEPIYEEIG
351 GAADPQRTRE NWSTRLWNQI REALVSLGGM ILSILGSILH RLRIARHAAA
401 EAVGRCCTCR GEECTSSSEED SMSVGSPEI DETERTGSPH DVPRRNGSPR
451 EDSPLMNALV GWAHKGAKT KESSESSTPE ISISAPIVRG WSQDSSVSFI
501 VMEDDHIFYD VPRRKDGIYD VPSSPRWSPA RELEEDVFGD YEVPTSAEP
551 SKDKNIYMTF RLATPAIYDL PSRPGSSGSS RSPSSDRVRS SSPNRRGVPL
601 PPVPSAMSE EGSYEDMSG ASGAGESDYE DMSRSPSPRG DLDEPIYANT
651 PEDNPFTQRN IDRILQERSG GASASFVEPI YDEIPWIHGR PPATLPRPEN
701 TLTNVSLRVS PGFGEVRAA LLSSEVSAMV VEAESIVPPT EPGDGESEYL
751 EPLGLVATT KILLQKGWPR GESNA*

The cp6756 nucleotide sequence <SEQ ID 260> is:

1 ATGGCATCAG GAATCGGAGG ATCTAGTGGA TTAGGAAAGA TTCCACCTAA
51 AGATAATGGG GATAGAAGTC GATCGCCCTC TCCTAAGGGA GAACCTGGCA
101 GCCACGAGAT TTCCCTGCCT CCTCAAGAAC ATGGAGAGGA AGGAGCTTCA
151 GGATCTTCGC ATATACATAG CAGTTCTCTT TTTCTACCAG AAGATCAGGA
201 GTCTCAGAGC TCTTCTTCGG CAGCTTCTAG CCCGGGATTT TTTTCTCGCG
251 TACGTTCTGG GGTAGACAGG GCCTTAAAT CATTTGGCAA CTTTCTTCC
301 GCAGAGTCTA CGAGTCAAGC GCGTGAACG CGACAAGCTT TTGTTAGATT
351 ATCAAAAACC ATCACCAGCG ATGAGAGACG GGATGTCGAT TCATCAAGTG
401 CTGCTGCTAC AGAAGCCCGA GTGGCAGAGG ACGCGAGTGT TTCAGGCGAA
451 AATCCTTCTC AGGGGGTTCC AGAAACCTCT TCTGGACCAG AACCTCAGCG
501 TTTATTTTCT CTTCCTTCAG TAAAAACA GAGCGGTTTG GGTCCGTTGG
551 TACAGACAGT TCGCGATCGC ATAGTACTTC CTAGTGGGGC TCCACCTACA
601 GACAGCGAGC CTTTAAGTCT CTACGAGCTA AACCTCCGTT TGAGTAGTTT
651 ACGTCAGGAG CTCTCTGACA TACAAAGTAA TGATCAGTTG ACTCCAGAGG
701 AAAAAGCAGA AGCCACAGTT ACCATACAAC AGCTGATCCA AATTACAGAA
751 TTCCAATGCG GCTATATGGA GGCAACACAA TCTTCGGTAT CTCAGCAGA
801 AGCTCGTTT AAGGGGGTAG AAACAGTGA TGAGATCAAT TCCCTCTGTT
851 CAGAACTGAC AGATCCTGAG CTTCAAGAAC TCATGAGTGA TGGAGACTCT
901 CTTCAAAACC TATTAGATGA GACTGCCGAC GATTTAGAAG CTGCTTTGTC
951 CCATACTCGA TTGAGTTTCT CTTTAGACGA TAATCCAAC CCGATAGACA
1001 ATAATCCAAC TCTGATTCTT CAAGAAGAGC CTATTATGA GGAAATCGGA
1051 GGAGCTGCAG ATCTCAAAG AACTCGGGAA AACTGGTCTA CAAGATTATG
1101 GAATCAGATT CGCGAGGCTC TGGTTTCTCT TTTAGGAATG ATTTAAGCA
1151 TTCTAGGGTC CATCTGCAC AGGTTCGCTA TTGCTCGTCA TGCAGCTGCT
1201 GAAGCAGTGG GTCGTTGTTG CACGTGCCGA GGAGAAGAGT GTACTTCTTC
1251 TGAAGAGGAC TCGATGTCGG TGGGGTCTCC TTCAGAAATT GATGAAACTG
1301 AAAGAACGGG CTCTCCGCAT GACGTTCCAC GCAGAAATGG AAGTCCACGT
1351 GAAGATTCTC CATTGATGAA TGCCTTAGTA GGATGGGCAC ATAAGCACGG
1401 TGCTAAAACC AAGGAGAGTT CAGAATCAAG TACCCCGGAA ATTTTCGATT
1451 CTGCTCCCAT AGTGAGAGGT TGGAGTCAAG ACAGTTCCGT CAGTTTATT

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1501 GTTATGGAAG ATGATCATAT TTTCTATGAT GTTCCTCGTA GAAAAGATGG
1551 AATCTATGAC GTTCCTAGTT CCCCTAGATG GAGTCCTGCG CGAGAGTTGG
1601 AAGAGGATGT TTTTGGAGAT TATGAAGTTC CTATAACCTC TGCTGAACCA
5 1651 TCTAAAGACA AGAACATCTA CATGACACCT AGATTAGCAA CTCCTGCTAT
1701 CTATGATCTT CCTTCACGTC CAGGATCGTC TGGAGGCTCA CGTTCTCCGT
1751 CTTCAGATCG CGTACGAAGC AGCTCACCAA ATAGACGGGG TGTGCTCTTT
1801 CCTCCAGTTC CTTACCTGTC TATGAGTGAG GAGGGGAGCA TTTATGAGGA
1851 TATGAGCGGT GCTTCAGGTG CAGGTGAAAG TGATTATGAA GATATGAGCC
10 1901 GTTCCCCCTC TCCTAGAGGC GACTTGGATG AACCATATA TGCTAATACT
1951 CCTGAAGATA ATCCATTTC TACAGAGAAAT ATAGATAGAA TTTTACAGGA
2001 GAGGTCAGGC GGTGCTTCCG CTTCTCCTGT AGAGCCTATT TATGATGAGA
2051 TCCCATGGAT TCATGGCAGG CCCCTGCTA CACTTCCAAG ACCCGAGAAT
2101 ACATTGACTA ATGTTTCGCT TAGAGTGAGC CCAGGGTTTG GACCAGAAGT
2151 AAGAGCCGCT TTGCTTAGCG AGAGCGTGAG TGCTGTTATG GTCGAAGCAG
15 2201 AGAGTATTGT TCCTCCAACA GAGCCGGGGG ACGGAGAATC AGAATATCTA
2251 GAGCCCTTAG GGGGACTTGT AGCTACAACG AAAATCTTAC TACAAAAAGG
2301 ATGCCTCGT GGAGAGTCGA ATGCTTAG

```

The PSORT algorithm predicts inner membrane (0.3994).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 130A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 130B) and for FACS analysis.

These experiments show that cp6756 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 131

The following *C.pneumoniae* protein (PID 4376761) was expressed <SEQ ID 261; cp6761>:

```

1 MTVAEVKGTG KLVCLGCRVN QYEVQAYRDQ LTILGYQEV L DSEIPADLCI
51 INTCAVTASA ESSGRHAVRQ LCRQNPTAHI VVTGCLGESD KEFFASLDRQ
101 CTLVSNKEKS RLIEKIFSYD TTFPEFKIHS FEGKSRAFIK VQDGCNSFCS
151 YCIIPYLRGR SVSRPAEKIL AELAGVVDQG YREVVIAGIN VGDYCDGERS
30 201 LASLIEQVDR IPGLIERIRIS SIDPDDITED LHRAITSSRH TCPSSHLVLQ
251 SGSNSILKRM NRKYSRGDFL DCVEKFRASD PRYAPTDDVI VGFPGESDQD
301 FEDTLRIIED VGFLKVHSFP FSARRRTKAY TFDNQIPNQV IYERKKYLAE
351 VAKRVGQKEM MKRLGETTEV LVEKVTGQVA TGHSPYFEKV SFPVVGTVAI
401 NTLVSVRLDR VEEELIGEI V*

```

The cp6761 nucleotide sequence <SEQ ID 262> is:

```

1 ATGACGGTTG CGGAAGTCAA AGGAACATTT AAGCTGGTCT GTTTAGGCTG
51 TCGGGTGAAT CAGTATGAGG TCCAAGCATA TCGCGACCAG TTGACTATCT
101 TAGGTTACCA AGAGGTCCTG GATTCTGAAA TCCCTGCAGA TTTATGCATA
151 ATCAATACGT GTGCTGTAC AGCTTCTGCT GAGAGTTCGG GTCGTCATGC
40 201 TGTGCGTCAG TTATGTCGTC AGAACCCTAC AGCACATATT GTTGTCACAG
251 GTTGTTTGGG GGAATCTGAC AAAGAGTTTT TTGCTTCTTT GGATCGGCAA
301 TGCACACTTG TTTCCAATAA AGAAAAATCC CGACTTATAG AAAAAATTTT
351 TTCCTATGAT ACGACCTTCC CTGAGTTCAA GATCCATAGT TTTGAGGGAA
401 AGTCTCGAGC TTTTATTAAA GTTCAAGATG GCTGTAATTC TTTTGTCTCG
45 451 TACTGCATTA TTCCTTATTT GCGGGGCGT TCGGTTTCTC GTCCTGCTGA
501 GAAGATTTTA GCTGAAATCG CAGGGGTGT AGACCAAGGA TATCGCGAAG
551 TTGTAATTGC AGGAATTAAT GTTGGAGATT ATTGCGATGG AGAGCGTTCA
601 TTGACCTCTT TGATGAACA GGTGACC CGG ATTCTTGGAA TTGAGAGGAT
651 TCGAATTTCC TCTATAGATC CTGATGATAT CACTGAAGAT CTGCACCGTG
50 701 CCATCACCTC ATCGCGTCAC ACTTGTCTCT CGTCACACCT TGTTCTTCAA
751 TCGGGGTCGA ATTCAATTTT AAAGAGAATG AACCGGAAGT ATTCTCGCGG
801 AGATTTTTTA GATTGTGTAG AGAAGTTCCG TGCTTCTGAT CCTCGCTATG
851 CCTTTACTAC AGATGTGATT GTCGGATTTC CTGGAGAGAG TGATCAAGAT
901 TTTGAAGATA CTTTGAGAAT TATTGAAGAT GTAGGCTTTA TTAAGTGCA
55 951 TAGTTTCCCT TTCAGTGCTC GTCGTCGTAC TAAGGCATAT ACTTTTGATA
1001 ATCAGATTCC CAATCAGGTG ATCTATGAGA GGAAGAAGTA TCTTGTCTGAG
1051 GTTGCTAAGA GGGTAGGCCA GAAAGAGATG ATGAAGCGTT TAGGAGAGAC

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1101 TACAGAGGTG CTTGTTGAGA AAGTAACGGG GCAGGTTGCT ACGGGTCACT
1151 CTCCTTATTT TGAAGAGGTT TCTTCCCTG TTGTAGGAAC GGTAGCTATC
1201 AACACTCTAG TTTCTGTGCG TCTTGATAGG GTAGAGGAAG AAGGGCTGAT
1251 TGGGGAGATT GTATGA

```

5 The PSORT algorithm predicts inner membrane (0.1574).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 131A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 131B) and for FACS analysis.

10 These experiments show that cp6761 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 132

The following *C.pneumoniae* protein (PID 4376766) was expressed <SEQ ID 263; cp6766>:

```

15 1 MATSVPTSS TSVGRANSSN ERFTERTSRM YYAALVLGAL SCLIFIAMIV
51 IFPQVGLWAV VLGFLGCLL LSLAIVFAVS GLVLGKLEP SREATPPEIV
101 AQKEWTTQOD VLGNEYWRSE LISLFLRGDL HESLIVDSKD RSLDIDQSLQ
151 NILKLEPLST TSLSLKKDCV HINIILHLVR QWNLGVDLS PEVTAHAEL
201 LLFLIEEQYV SPDILKLIRY GDALQATSPL MDWADSGSFS VDADGVFSCR
251 REECSPEDAL AQFDLLALE NPDRFLKDS FLTYIWSSSF FEKFLHRHLE
301 SLQRKLPETA IDVARYBAQI QFSLRYFQK LDLINAMSLD WGYNCAEGEK
20 351 CYESANQRLD NLFIAFSSSV PAMKRLFDKY GSVVRVDRRQ IREQILSNTE
401 ILENESGFLC SLYEYPLSYL IDWAVLLDCV RGTEISLEDQ ADYTVCLQGL
451 DSMLSQFASR LQSGQKVLNP RDVLSEQAQV MLVHGLAAQG VSPQGLKALM
501 YLTAVPQRMW LGALPLFESF PVFNRMKKEFL GESLGD*

```

The cp6766 nucleotide sequence <SEQ ID 264> is:

```

25 1 ATGGCAACCT CTGTTCTCTGT AACTTCATCT ACTTCTGTAG GAGAGGCTAA
51 CTCCTCCAAC GAAAGATTTA CTGAACGAAC ATCGCGAATG TATTACGCAG
101 CTTTAGTCCT AGGGGCTTTG AGCTGTTTAA TTTTATTGTC TATGATTGTC
151 ATTTTCCAC AGGTCGGATT GTGGGCTGTG GTCCTCGGTG TTGCTCTTGG
201 ATGTTTACTT TTAAGCTTAG CTATCGTTT TGTCTCTCC GGTCTCGTTT
30 251 TAGGCAAGAC TTTAGAACCT AGTCGAGAAG CGACTCCTCC AGAAATGTGT
301 GCGCAAAAGG AGTGGACTAC ACAACAAGAT GTCTTAGGGA ATGAGTATTG
351 GCGTTCGAG TTGATTTCTT TGTCTTACG AGGGGATCTC CACGAATCTC
401 TGATTTGTTGA TTCTAAGGAT CGATCTTTAG ATATTGATCA GAGTTTACAA
451 AATATATTGA AACTTGAGCC CCTATCTACG ACCTTTTCGC TGTAAAGAA
35 501 AGATTTGTGC CACATCAATA TCATTTTACA TTTAGTGAGA CAGTGGAACT
551 TACTGGGAGT GGATCTTAGT CCTGAAGTCA CTGCGCACGC CGAGGAACCT
601 CTACTCTTTT TGATAGAAGA GCAGTATTAC TCTCTGATA TTTTGAAATT
651 GATTGCTTAC GGAGATGCTT TACAAGCAAC GTCTCCTTTG ATGGATTGGG
701 CAGATTTCAG TTCTTTTAGT GTAGACGCAG ACGGGGTATT TAGCTGTCGC
40 751 AGAGAAGAAT GTTCTCTCTGA GGATGCTTTG GCGCAATTCT ATCTTCTTTT
801 GCGGTTGGAA AATCCCGACA GACGCTTCTT AAAGGATTCT TTTCTTACCT
851 ACATTTGGTC GTCCTCATTT TTTGAGAAGT TTTTACATCG CCATCTAGAG
901 AGCTTGCAAA GAAAGCTCCC AGAGACAGCG ATCGATGTCG CCCGCTATGA
951 AGCACAAATA CAAACATTTT TCTCTCGCTA TTTTCAGAAG CTCGATTTGA
45 1001 TAAACGCAAT GTCTTATAGT TGGGGATATA ACTGTGCTGA GGGAGAAAAA
1051 TGTTATGAGA GCGCAAAATCA AAGATTAGAC AACCTATTTA TTGCTTTTTC
1101 TTCTTCTGTT CCTGCTATGA AGCGGCTCTT TGACAAATAT GGTCTCTGTG
1151 TACGGGTAGA TCGTAGGCAG ATTCTGTAGC AGATTCTTTC GAACACTGAA
1201 ATCTTAGAAA ATGAGTCAGG GTTCTCTGTC AGTTTGTATG AATATCCTTT
50 1251 ATCCTATTGT ATAGATTGGG CTGTTTGTCT AGACTGTGTT CGCGGTACCG
1301 AAATCTCTCT AGAAGATCAG GCCGATTACA CCGTTTGTCT GCAAGGCTTG
1351 GATTTCTATGT TATCTCAATT TGCGAGTCGT TTACAGTCTG GACAAAAAGT
1401 ATTGAATCCT AGAGATGTTT TAAGTGAACA GGCTGCGGTT ATGCTTGTTC
1451 ATGGCTTGGC AGCACAGGGC GTGTCGTTTC AAGGATTGAA AGCTTTGATG
55 1501 TATTTGACAG CCGTTCCCCA AAGAATGTGG TTAGGAGCAT TGCCTTTATT
1551 TGAATCTTTT CCTGTCTTTA ATCGGATGAA AGAATTTCTT GGGGAATCTC
1601 TGGGAGACTA G

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The PSORT algorithm predicts inner membrane (0.6158).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 132A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 132B) and for FACS analysis.

- 5 These experiments show that cp6766 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 133

The following *C.pneumoniae* protein (PID 4376804) was expressed <SEQ ID 265; cp6804>:

```

10      1 MSNQLQPCIS LGCVSYINSF PLSLQLIKRN DIRCVLAPPA DLLNLLIEGK
      51 LDVALTSSLG AISENLGYVP GFGIAANQRI LSVNLYAAPT FFNSPQPRIA
     101 ATLEBRSSIG LLKVLCRHLW RIPTPHILRF ITTKVLQPTP ENYDGLLLIG
     151 DAALQHPVLP GFVTYDLASG WYDLTKLPFV FALLHSTSW KEHPLPNLAM
     201 EEALQQFESS PEEVLKEAHQ HTGLPPSLLO EYALCQYRL GEEHYESFEK
     251 FREYYGTLYQ QARL

```

- 15 The cp6804 nucleotide sequence <SEQ ID 266> is:

```

      1 ATGTCTAACC AACTCCAGCC ATGTATAAGC TTAGGCTGCG TAAGTTATAT
     51 TAATTCCTTT CCGCTGTCCC TACAACTCAT AAAAAGAAAC GATATTCGCT
    101 GTGTTCTTGC TCCCCCTGCA GACCTCCTCA ACTTGCTAAT CGAAGGGAAA
    151 CTCGATGTTG CTTTGACCTC ATCCCTAGGA GCTATCTCTC ATAACTTGGG
    201 GTATGTCCCC GGCTTTGGAA TTGCAGCAAA CCAACGTATC CTCAGTGTA
    251 ACCTCTATGC AGCTCCCACT TTCTTTAACT CACCGCAACC TCGGATTGCC
    301 GCAACTTTAG AAAGTCGCTC CTCTATAGGA CTCTTAAAG TGCTTTGTCTG
    351 TCATCTCTGG CGCATCCCAA CTCCTCATAT CCTAAGATTG ATAACTACAA
    401 AAGTACTCAG ACAAAACCCCT GAAAATTATG ATGGCCTCCT CCTAATCGGA
    451 GATGCAGCGC TACAACATCC TGTACTTCCT GGATTGTAA CCTATGACCT
    501 TGCCTCGGGG TGGTATGATC TTACAAAGCT ACCTTTGTGTA TTTGCTCTTC
    551 TTCTACACAG CACCTCTTGG AAAGAACATC CCCTACCCAA CCTTGCATG
    601 GAAGAAGCCC TCCAACAGTT CGAATCTTCA CCCGAAGAAG TCCTTAAAGA
    651 AGCTCATCAA CATACAGGTC TGCCCTTTC TCTTCTTCAA GAATACTATG
    701 CCCTATGCCA GTACCGTCTA GGAGAAGAAC ACTACGAAAG CTTTGAAAAA
    751 TTCCGGGAAT ATTATGGAAC CCTCTACCAA CAAGCCCGAC TGTA

```

The PSORT algorithm predicts inner membrane (0.060).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 133A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 133B) and for FACS analysis.

These experiments show that cp6804 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 134

The following *C.pneumoniae* protein (PID 4376805) was expressed <SEQ ID 267; cp6805>:

```

40      1 MSSLLSCGRI EPTRVTCSLK TYLEDTSQNO LSTRLVASV IFLCALLIIL
     51 VCVALSSLIP SIMALATSFT VMGLILFVMS LLGDVAIISY LTYSTVTSYR
    101 QNKRAFEIHK PARSVYYEGV RHWDLGRSSL GTGEIPIVRT LFSPPQNHGL
    151 NHALAAKIFL FMEHFSPEPP NEPLVDWACL IRDFRPHVSS LCFVIEKQGS
    201 SLRTKEGNTI CEAPRSDYDA HFAMVDCYRL IHSKLIIEKM GLKNIDIIPS
    45  251 VMVREDYPSR PEGGYREGLL RMYGGKGAL*

```

The cp6805 nucleotide sequence <SEQ ID 268> is:

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```

1  ATGTCATCAC TACTGAGCTG CGGAAGAATA GAGCCGACTC GGGTTACCTG
51 TAGCTTAAAG ACGTATCTTG AGGATACGAG TCAGAATCAG TTGAGCACAC
101 GTCTAGTTTCG GGCAAGTGTC ATCTTTTAT GCGCATGTGT GATCATTTTG
5  151 GTTGTGTGG CCCTCTCTAG TTTGATTCCA AGCATTATGG CCTTGGCGAC
201 CTCTTTTACG GTAATGGGGT TAATCTTTT TGTGATGTCA CTTCTTGGTG
251 ACGTTGCAAT TATAAGTTAT CTTACTTATA GCACGTGTAC GAGTTACCGG
301 CAAAATAAGA GAGCTTTTGA GATTACAAG CCCGCTCGCT CCGTTTACTA
351 CGAGGGGGTC CGCCATTGGG ATTTAGGACG ATCATCTTTA GGCACAGGCG
10  401 AGATTCCTAT AGTAAGGACG TTATTCTCTC CATTCAGAA CCATGGTCTT
451 AACCATGCCT TAGCTGCTAA AATTTTCTTA TTTATGGAGC ATTTAGCCCC
501 TGAGCCACCG AACGAGCCTT TGGTGGATTG GGCCTGTTTG ATTCGGGATT
551 TTAGGCCTCA CGTCAGTTCT TGTGCTTTG TTATTGAAAA ACAAGGGTCA
601 TCCTGAGGA CTAAGGAAGG CAATACGATT TGTGAGGCTT TCCGCTCTGA
651 TTACGACGCC CATTTTGCTA TGGTAGATTG CTACCGGTTG ATCCACTCTA
15  701 AGTTGATTAT AGAGAAAATG GGATTGAAGA ATATCGATAT CATTCCGAGT
751 GTCATGGTTC GTGAAGATTA TCCTAGCCGT CCTGGGGAGG GCTATCGCGA
801 AGGCCTATTA CGTATGTATG GTGGCAAGGG GGCTCTGTGA

```

The PSORT algorithm predicts inner membrane (0.711).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 134A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 134B) and for FACS analysis.

These experiments show that cp6805 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 135

The following *C.pneumoniae* protein (PID 4376813) was expressed <SEQ ID 269; cp6813>:

```

1  MSGPSRTESS QVSVLSYVPR DKEIAPKKQF TIAKISTLAI LASLALGALV
51 AGISLTIVLG NPVFLALLIT TALFSVVTFL VYHQMTSKVS SNWQKVLEQN
101 FKPLGKAWQE KNVDCYSNEM QFYNNHLNPK FKVAIQTDAS QPFQPTFLTG
151 LRVIEKNQST GIIFNPVGPT NLIDNATNL STILYSTLKD KSVWDTCKQR
30  201 EGGPAKGEDP FSPTFVRVVK LPNEALDQTF NLNLSAEKK SILPTFLGHV
251 CGPRSEELPN QQEYYRQALL AYENCLKAAI ESHAATVALP LFTSVYEVPP
301 EEILPKEGTF YWDNQTAFC KRALLDAIQN TALRYPQRS LVLQLDPFNT
351 IESQSRSEE*

```

The cp6813 nucleotide sequence <SEQ ID 270> is:

```

35  1  ATGTCAGGAC CCTCAGTAC TGAGAGCTCT CAAGTTTCTG TACTATCCTA
51  TGTGCTCGG GATAAAGAAA TTGCTCCTAA AAAACAGTTT ACCATAGCAA
101 AAATATCCAC TCTTGCAATC CTAGCTTCTT TAGCTTTAGG AGCTTTGGTG
151 GCTGGAATCT CTTTAACGAT AGTATTAGGG AACCTGTAT TTTTGGCTCT
201 TCTCATTACC ACGGCCCTCT TCTCAGTTGT AACCTTCTTA GTCTACCACC
40  251 AAATGACCTC AAAGGTATCT TCTAACTGGC AGAAAGTTCT AGAGCAAAAC
301 TTCAAGCCTT TGGGAAAAGC GTGGCAAGAA AAAAAAGTAG ACTGCTACTC
351 AAACGAGATG CAATTTTACA ATAATCACCT GAACCTAAG TTCAAGGTAG
401 CGATACAAAC AGATGCGTCT CAACCATTTT AGCCTACTTT CTTAACTGGA
451 CTTAGAGTGA TCGAAAAAAA TCAATCCACA GGGATCATCT TTAATCCCGT
45  501 AGGCCCAACG AATCTGATCG ACAACACTGC AACGAACCTC TCTACTATCC
551 TTTACTCCAC CCTAAAAGAT AAAAGCGTGT GGGATACATG CAAGCAACGC
601 GAAGGGGGTC CCGCAAAAGG AGAAGACCCC TTTTCCCCTA CCGAAGTGAG
651 AGTAGTAATA CTTCCAAACG AAGCTCTAGA TCAAAAGTTT AATCTAAATT
701 TAAGCTCTGC AGAAAAGAAA AGTATPCTTC CGACCTTTTT AGGCCACGTA
50  751 TGCGGCCCTA AATCTGAAGA GTTACCAAAT CAGCAAGAAT ATTATCGCCA
801 AGCTTTACTA GCGTACGAGA ACTGCCTTAA AGCAGCTATA GAAAGTCATG
851 CAGCAATCGT TGCTCTTCCT CTCTTTACTT CGGTCTATGA AGTGCCTCCA
901 GAAGAGATTG TTCTTAAAGA AGGCACTTTC TATTGGGACA ACCAACTCA
951 AGCGTTTTGC AAACGCGCTT TATTGGACGC TATTCAAAAT ACGGCCCTAC
55  1001 GCTATCCTCA AAGATCTTTA CTTGTATATC TCCAAGATCC TTTTAATACT
1051 ATAGAATCAC AAAGTCGTTT TGAGGAGTAA

```

The PSORT algorithm predicts inner membrane (0.4291).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 135A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 135B) and for FACS analysis.

- 5 These experiments show that cp6813 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 136

The following *C.pneumoniae* protein (PID 4376844) was expressed <SEQ ID 271; cp6844>:

```

10      1  MWRVVLRLFI  IFILGRAVFP  LRASESFSWE  TSTCLTVLGI  PFIDIILTNN
      51  EDFVAQCGLQ  IGTISSTNNA  KIKEIFLIYK  EKFPESISF  KRKEPLNLSQ
     101  SHLSDLGLIC  MRNGETYABG  MANKENGPAL  KQPKDLRLVL  RCPNQPDLL
     151  YSEKRAEKGI  ETNTCLCNQG  YTLLDGQLIL  YGDSIEKFLK  ETKRKNNHTL
     201  VDLCDQSQVVT  TFLGRFWSLL  NYVQVLFLE  DSAKILAGIP  DLAQATQLLS
     251  HTVPLLFYIT  NDSIHIEQG  KESSFTYNQD  LTEPILGFLF  GYINRGSMFY
     301  CFNCAQSSLG  ET*
```

The cp6844 nucleotide sequence <SEQ ID 272> is:

```

20      1  ATGTGGCGCG  TTGTCTCAG  ATTCCTTATA  ATTTTATCT  TGGGAAGAGC
      51  CGTCTTCCCT  CTAAGAGCTT  CAGAAAGCTT  CTCTGGGAA  ACATCGACCT
     101  GTTTAACAGT  GCTAGGGATT  CCTTTCATAG  ATATTATCCT  CACAACGAAT
     151  GAGGACTTTG  TTGCCAGTG  CGGCCTGCAA  ATAGGAACCA  TTTCTTCGAC
     201  TAATAACGCA  AAAATAAAG  AAATTTTTF  GATATATAAG  GAAAAATTTC
     251  CAGAAGCCTC  TATCAGTTTC  AAACGAAAAG  AACCTCTAAA  CCTTTCCCAA
     301  TCCCATCTCT  CCGATTTAGG  TATTTTATGT  ATGCGTAACG  GAGAAACTTA
     351  CGCTGAGGGA  ATGGCAAATA  AAGAAAACGG  ACCCGCTCTA  AAACAACCCA
     401  AGGATCTAAG  ATTAGTTTTA  CGTTGTCTTA  ACCAACCAGA  TACCCTGCTC
     451  TACTCGGAAA  AAGAAGCAGA  AAAGGGCATA  GAAACAAATA  CTTGCCTATG
     501  CAATCAGGGA  TACACACTCC  TGGATGGGCA  ATTGATCTCT  TACGGGGATA
     551  GTATAGAAAA  GTTCTGAAA  GAGACCAAAA  GAAAGAATAA  CCACACGCTT
     601  GTTGATCTTT  GTGACTCACA  AGTCGTGACC  ACGTTCCTCG  GTCGCTTTTG
     651  GTCTCTTCTA  AACTACGTTT  AAGTTCCTTT  CCTATCTGAA  GACTCCGCTA
     701  AAATCTTTCG  GGGCATCCCA  GACCTAGCTC  AAGCTACGCA  ATTGCTTTCC
     751  CACACCGTAC  CTTTCTCTTT  TATTTATACC  AACGATCTTA  TTCACATCAT
     801  AGAACAAGGC  AAAGAAAGTA  GTTTTACCTA  TAACCAAGAT  TTAACAGAGC
     851  CCATTTTAGG  ATTTCTCTTT  GGTTACATAA  ATCGCGGCTC  TATGGAATAC
     901  TGCTTTAATT  GTGCACAGTC  TTCATTAGGA  GAAACCTAA
```

The PSORT algorithm predicts inner membrane (0.1786).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 136A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 136B) and for FACS analysis.

- 40 These experiments show that cp6844 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 137

The following *C.pneumoniae* protein (PID 4377201) was expressed <SEQ ID 273; cp7201>:

```

45      1  VLVGICPSLY  PEHPRSFYRK  VSGDIGSRFD  DRGFVNSGVE  TLPYSSGSFG
      51  IFWISPTDPT  FNFAIVNIFM  RTAGINEVSR  PMTQDTETSL  IEMRDLSEQQ
     101  EANNNTDSLEQ  EESLMGIVGH  TVGGVSMVT  SSPNIFYRIQ  TLLGLPETLA
     151  EARENPTFPN  STIDSLAEIM  MNLVRISDAV  SIFWIFPIVD  TTYNGVLLAV
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201 CIGFFGINGI CSTFLMLTNP RSRDRWRNL RIMVLCYRSL GSGMNLFDLS
251 NNVMAARRH VTSCVALYA MVTLFGWTVA IQDALQYGFP SVRDAFYRYC
301 LRHRYCLTQR NEDSLQTTGT RFQVTRTHLE DQQMVASILN LSVFGLFFGF
351 VGLMTTFGGL EISPSRWDA ANNRTVGIF*

```

5 The cp7201 nucleotide sequence <SEQ ID 274> is:

```

1 GTGCTCGTTG GTATCTGTCC TTCTCTATAT CCAGAACATC CTCGCTCCTT
51 TTATTATCGT GTTCTCGGAG ATATAGGCTC CCGATTGAC GATAGAGGAT
101 TTGTAAACTC TGGAGTCGAA ACCCTGCCAT ACTCTTCAGG CAGCTTTGGG
10 151 ATTTTTTTGA TCTCGTTTAC GGATCCACA TTTAAATTTG CTATCGTAAA
201 TACCTTTATG CGAACTGCAG GGATCAATGA AGTCTCTAGA CCCATGACAC
251 AAGATACAGA AACTTCATTG ATAGAAATGA GAGACCTAAG TGAACAACAA
301 GAAGCGAATA ACACAGATTC TTTAGAGCAA GAAGAGAGCT TAATGGGTAT
351 TGTAGGACAT ACTGTGGGAG GAGTTTCCAT GACCGTGACC TCCAGTCCAA
401 ATATCTTTTA TCGTATACAA ACACTTCTGG GACTGCCAGA GACTCTTGCA
15 451 GAAGCTGAAG AAAATCCTAC CTTCCCAAAT TCTACTATAG ATAGCCTTGC
501 AGAAATAATG ATGAACCTCG TAAGGATCTC TGATGCTGTC TCTATTTTCT
551 GGATTTTTC TATCGTAGAT ACTACATATA ATGGAGTTTT ATTAGCCGTC
601 TGTATCGGCT TCTTCGGAAT CAATGGGATT TGTTCACCGT TCCTTATGCT
651 TACGAATCCA CGCTCTCGTC GAGATAGATG GAGGAATTTA CGCATCATGG
20 701 TCTTTTGCTA TCGTTCTTTG GGAAGCGGAA TGAATCTCTT TGATCTTAGC
751 AATAATGTGC GCATGGCAGC ACGTAGGCAT GTGACATCAT GTACAGTAGC
801 TCTCTATGCT ATGGTCACTC TATTTGGATG GACAGTAGCA ATACAAGATG
851 CTTTGCAATA TGGTTTCCCT AGCGTTCGGG ATGCCCTCTA TAGATATTGC
901 TTACGCCACA GATATTGCTT AACTCAAAGA AACGAAGACT CTCTGCAAAC
25 951 TACAGGAACG CGCTTTCAGG TTACCGGTAC ACATCTAGAA GATCAACAGA
1001 TGGTGGCTTC TATTTTGAAT TTGAGTGTTC TTGGGCTCTT TTTTGGATTTC
1051 TGAGGGCTAA TGACCACGTT TGGAGGATTA GAAATCTCAC CATCTTGTCG
1101 GTGGGATGCA GCAAATAACC GAACGGTAGG TATTTTMTAG

```

The PSORT algorithm predicts inner membrane (0.3102).

30 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 137A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 137B) and for FACS analysis.

These experiments show that cp7201 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

35 Example 138

The following *C.pneumoniae* protein (PID 4377251) was expressed <SEQ ID 275; cp7251>:

```

1 MAPIHGSNAF VEDILHSHPS PQATYFSSTR AQKLHEFKDR HPVLTRIASV
51 IIRIFKVLIG LIILPLGIYW LCQTLCTNSI LPSKNLLKIF KKQPNKTKLK
101 TNYLHALQDY SSKNRVASM RVPILODNVL IDTLEICLSQ APTNRWMLIS
40 151 LGSDCSLEEI ACKEIFDSWQ RFAKLIGANI LVYNYPGVMS STGSSSLKDL
201 ASAHNICTRY LKDKQGGPGA KEIITYGYSL GGLIQABALR DQKIVANDDT
251 TWIAVKDRCP LFISPEGFHS CRRIGKLVAR LFGWGTKAVE RSQDLPCLEI
301 FLYPTDSLRR STVRQNKLLA PELTLAHAIAK NSPVVQNKKEF IEVRLSSDID
351 PIDSKTRVAL ATPILKKLS*

```

45 The cp7251 nucleotide sequence <SEQ ID 276> is:

```

1 ATGGCTCCAA TTCACGGAAG TAATGCGTTT GTTGAGGATA TTTTACATTCT
51 CCACCCCTTCT CCACAAGCGA CTTATTTTTC TTCAACACGC GCCCAAAAAC
101 TTCATGAGTT TAAAGACAGG CATCCCGTGC TTACACGGAT TGCTTCTGTA
151 ATTTATAAAA TTTTAAAGT TCTGATAGGG CTGATCATCC TTCCCTTAGG
50 201 AATCTACTGG CTATGTCAAA CGCTTTGTAC AAACCTCGATT CTCCCTTCCA
251 AGAATTTTAT AAAAATTTTC AAGAAGCAAC CCAACACTAA AACCTTAAAA
301 ACTAATTATT TGCATGCTTT GCAAGATTAT TCCTCGAAAA ACCGCGTTGC
351 TCTCATGAGA CGAGTTCCTA TCCTCCAGGA TAATGTCTCT ATCGACACTT
401 TGGAAATATG CCTTTCACAA GCACCTACGA ATCGTTGGAT GCTCATTTCT
55 451 TTAGGAAGTG ACTGTAGCTT GGAAGAAATC GCTTGTAAGG AGATCTTTGA

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501 TTCTTGCCAA AGATTGCGCA AGTTGATAGG GGCCAATATA CTCGTTTATA
 551 ACTACCCCGG AGTCATGTCC AGCAGAGGGA GCAGCAGCCT AAAGGACCTA
 601 GCATCAGCTC ATAATATTG TACAAGATAC CTTAAAGATA AAGAACAGGG
 651 CCCTGGAGCA AAAGAAATCA TTACCTATGG GTACTCCCTA GGAGGTTTGA
 5 701 TACAAGCAGA AGCATTGCGA GACCAGAAGA TTGTTGCAAA CGATGATACT
 751 ACTTGATAG CAGTCAAAGA TAGGTGTCCT CTCCTTATAT CTCCAGAAGG
 801 TTTCCACAGT TGCAGACGCA TAGGAAAGCT AGTAGCTCGT CTTTTTGGCT
 851 GGGGGACCAA AGCCGTAGAG AGAAGCCAAG ACCTTCCCTG CCTAGAAATT
 901 TTTCTCTATC CTACGGATTC CTTACGAAGA TCAACAGTCA GACAGAACAA
 10 951 GCTCTTAGCA CCTGAACCTA CTCTCGCTCA TCGGATAAAA AATAGTCCCT
 1001 ATGTTCAAAA TAAAGAATTT ATAGAAGTAC GATTATCGTC TGATATCGAT
 1051 CCCATCGACA GCAAAACAAG AGTGGCTCTT GCCACACCA TTTTGAAAAA
 1101 GCTCTCTTAG

The PSORT algorithm predicts inner membrane (0.4545).

15 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 138A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 138B) and for FACS analysis.

These experiments show that cp7251 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

20 Example 139

The following *C.pneumoniae* protein (PID 4377288) was expressed <SEQ ID 277; cp7288>:

1 MHMSNPISLF SPAELIAKYN LIPKTSPIYP RRELIILEE NACQTRLTNV
 51 AQVLHPSSLF SMSKKILNPC GCSGGPLCWV ILNILAFIIT SVLFIILLPV
 101 NLIVAGLRLF MPLPPKKIVE DLSEPTTEET NEVIQPFIFA LQALLFEDNK
 25 151 LRSFKIVEQS VGKAPLPNPF LNRLVAISPQ ESQEMARKIP DLCSQLKKVL
 201 KSLGVLTPPEW KHMLKYFEBGL KNEHDSNPDK KTFPILIKLL IEALTGKSSL
 251 PKTPSTKERM QAALFIASSC RTCKPTWGEV ITRSLNRLYS IANEGDNQLL
 301 IWVQBFKERE LMSIQDGDGA KEYRFAAQQH GERYTEAIEQ VLRNESAAKL
 351 QWHVINIMKF FHGKNLGLVT EHLQDTLGL TLRTQTTVDTH QGREDDADLSA
 30 401 ALFLNKLYLNS GNQLVNSVFK SMQKADPETK ALIREFALDI LYASLRLPQT
 451 SAHTEVFSTL LMDPETYEPN KACIAYLLYV LKIIEL*

The cp7288 nucleotide sequence <SEQ ID 278> is:

1 ATGCATATGT CTAACCCCAT CTCTTTGTTT TCCCCTGCAG AGTTAATAGC
 51 AAAGTACAAT TTAATTCCAA AAACCTCGCC GATTATCCT CGGAGGACGG
 35 101 AACTTATTAT CTTGGAAGAA AATGCGTGT CAAACACGCT AACCAACGTG
 151 GCTCAGGTCC TACATCCTTC TAGCCTATTC AGTATGTCAA AAAAAATACT
 201 GAATCCCTGC GGGTGCTCTG GTGGTCCCTT ATGTTGGGTG ATTCTCAACA
 251 TCCTAGCATT TATTATTACT TCAGTACTGT TTATCATTC TTTACCGGTG
 301 AATCTCATCG TAGCAGGTCT TCGTCTCTTC ATGCCTCTTC CCCCTAAAAA
 40 351 AATCGTAGAG GATTAAAGTG AACCTACTAC TGAAGAAACG AATGAGGTCA
 401 TTCAACCTTT CATTTTCGCT TTGCAAGCGT TGCTTTTGA GGATAACAAA
 451 CTTCGCTCTT TTAATAATGT TGAACAAAGT GTAGGCAAAG CACCCTTACC
 501 TAATCCCTTT TTAATAGAC TAGTAGCAAT TTCGCCGCAA GAAAGCCAAG
 551 AAGCCATGCG GAAGATTCCG GATCTATGCT CACAACGAA AAAAGTATTA
 45 601 AAGTCTCTAG GCGTGCTAAC TCCAGAATGG AAGCACATGC TGAAGTACTT
 651 TGAGGGACTG AAAAACGAAC ATGATAGTAA TCCTGATAAA AAGACGTTCC
 701 CAATATTGAT CAAGCTCCTC ATAGAAGCTC TTAAGTGAAG GTCTCTTTTA
 751 CCCAAAACCT CTAGTACAAA GGAAGAAATG CAAGCGGCCT TATTATTGTC
 801 AAGTCTTTCG AAGACTTGTA AGCCGACTTG GGGAGAAGTC ATAACCAGAT
 50 851 CTCCTAACAG ACTCTATAGT ATAGCTAATG AAGGAGACAA TCAGCTTCTG
 901 ATTTGGGTTC AAGAGTTTAA AGAAGGAGAG CTGATGTCCA TCCAAGATGG
 951 TGATGATGCT GAAGAGTATC GGTTCGCGC TCAGCAACAC GGTGAGCGTT
 1001 ACACAGAGGC AATAGAACAA GTTCTACGAA ACGAGTCAGC AGCCAAACTA
 1051 CAATGGCATG TGATCAACAC TATGAAATTC TTCCATGGGA AAAATCTCGG
 55 1101 TCTAGTTACA GAACACCTAC AAGATACTCT CGGCGCCCTA ACTTTACGTC
 1151 AAATACAGT GGACACACAT CAAGGCAGAG AAGACGCTGA TTTGTACGCT
 1201 GCTCTTTTCC TAAATAAGTA TTTAAATCTT GGAATCAAC TTGTTAATAG

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1251 CGTCTTTAAA TCCATGCAAA AAGCAGATCC AGAAACCAAA GCTTTAATCC
1301 GTGAGTTTGC TCTAGATATA TTATATGCAT CCTTACGGCT TCCTCAAACT
1351 TCCGCTCATA CCGAGGTCTT TTCTACACTC TTAATGGACC CAGAGACCTA
1401 TGAACCTAAT AAAGCTTGTA TCGCCTACTT GCTCTATGTA TTAAAGATCA
1451 TCGAAGTATA A

```

The PSORT algorithm predicts inner membrane (0.5989).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 139A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 139B) and for FACS analysis.

- 10 These experiments show that cp7288 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 140

The following *C.pneumoniae* protein (PID 4377359) was expressed <SEQ ID 279; cp7359>:

```

1  MPGSVSSPPL SPVIVRERV SSSGSDLIQP HAVLKISILI FALVTILGIV
51  LVLVSSALGA LPSLVLTVSG CIAIAVGLIG LGILVTRLIL STIRKVDAMG
101 YDAAVKEEQY LSRIRELESE NREIRDNRRA VEDQCAHLSE ENKDLRDPEY
151 LHGMTERLIA SLEIENQALV AENILKLDWN ASLSRDFRAY KQKFPLGALE
201 PWKEDIACIM EQNLFLKPEC IAMVKSLEPLE TQRLFLYPKG PQSLVNRFPAP
251 RSRFFQTPKY EYNSRNENED GKVAAVCARL KKEFFSAVLG ACSYEELGGI
301 CERAVALKET LPLPEAVYDT LVQEFNNLLT AESLWKEWCF YSYPYLRPYL
351 SVDYCKRLFV QLFEECLCLKL FTTGSPEDQA LVRLFSYYRN HIPAVLASFG
401 LPPPETGGSV FVLLPKQENL LWSQIEVLAT RYLKDTFVRN SEWTGSFEMM
451 FSYNEMCKEI SEGRIRFAED YETRHSEEFPS PSPLSEEGEG EEFLPPCSEE
501 EVSVLERPDL DVDSMWVWHP PVPKGPL*

```

25 The cp7359 nucleotide sequence <SEQ ID 280> is:

```

1  ATGCCAGGTT CTGTGTCATC ACCTCCTTTG TCTCCTGTAA TTGTCCGTGA
51  AAGGGTCCCA TCCTCTTCAG GATCCGACCT CATACAGCCT CATGCTGTTT
101 TAAAGATCTC CATCTAATTT TTTGCGCTTG TGACAATTTT AGGAATTGTT
151 CTTGTAGTGT TGTCTAGTGC TTTAGGAGCT CTTCTAGTGT TAGTTTTGAC
201 GGTTCCTGGT TGTATTCGAA TAGCTGTAGG CCTGATTGGT TTAGGGATTC
251 TTGTGACACG GCTGATTCTC TCTACGATCA GAAAAGTAGA TGCCATGGGT
301 TATGATGCTG CCGTCAAAGA AGAGCAGTAT TTGTACGTA TCAGAGAATT
351 AGAGTCTGAA AATAGAGAGA TTAGAGATAG AAATCGTGCT GTCGAAGATC
401 AGTGTGCCCA TTTATCCGAA GAGAACAAGG ACCTTAGGGA TCCCGAATAT
451 CTACATGGAA TGACTGAAAG GCTCATTCGG AGCTTAGAAA TAGAGAATCA
501 AGCTCTCGTA GCTGAGAACA TTCTTCTCAA AGACTGGAAT GCAAGCCTAT
551 CTAGAGATTT CCGCGCATAT AAGCAAAAAT TTCCTCTTGG GGCATTAGAA
601 CCCTGGAAG AAGATATTGC ATGTATCATG GAACAAAATC TCTTTTAAA
651 ACCGGAATGT ATCGCGATGG TTAAGTCTCT TCCATTAGAG ACGCAACGGC
701 TGTTTTATA TCCAAAAGGA TTTCACTCTT TAGTTAATCG ATTTGCTCCG
751 CGGTCTCGCT TTTTCCAGAC TCCAAAGTAT GAATATAACA GTAGGAATGA
801 AAATGAGGAC GGAAAGGTAG CCGCAGTGTG CGCCCGTTTG AAAAAAGAA
851 TCTTCAGTGC TGTTTTAGGA GCCTGTAGTT ACGAAGAACT AGGGGGCATT
901 TGTGAAAGAG CAGTAGCACT TAAAGAGACG TTGCCATTGC CTGAAGCTGT
951 CTATGATACC CTAGTTCAGG AGTTCCCAA TCTTCTTACT GCTGAGAGTT
1001 TATGAAAGA ATGGTGCTTC TATTCTTATC CCTACCTTCG TCCCTATCTT
1051 TCTGTGGATT ACTGTAAGAG GTTATTGTGA CAACTTTTGG AGGAACCTCTG
1101 CCTAAAGCTT TTTACAACGG GATCTCCAGA AGACCAAGCT TTGGTTTCGCC
1151 TTTTCTCTTA CTATAGGAAT CATATTCCCG CAGTCTTGGC CTCATTGGGT
1201 TTGCCCCCGC CTGAGACAGG GGGGTCTGTA TTTGTATTGC TACCAAAACA
1251 AGAAAACCTT CTTTGGAGTC AAATTGAGGT GCTGGCTACA AGGTATCTCA
1301 AAGATACCTT CGTGAGAAAC TCAGAAATGGA CGGGCTCTTT CGAGATGATG
1351 TTTTCTTATA ACGAGATGTG TAAGGAGATC TCCGAAGGAA GGATTCTGTT
1401 TGCTGAAGAC TATGAAACGA GGCATTCCGA AGAATCCCTT CCTTCCCTCTC
1451 TCTCTGAAGA AGGAGAGGGC GAAGAATTCC TTCCTCTTGG CTCTGAAGAA
1501 GAGGTTTCGG TTCTTGAGCG CCCAGATCTA GATGTAGACT CTATGTGGGT
1551 CTGGCATCCG CCGGTCCTTA AGGGACCTCT TTAA

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The PSORT algorithm predicts inner membrane (0.7453).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 140A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 140B) and for FACS analysis.

- 5 These experiments show that cp7359 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 141

The following *C.pneumoniae* protein (PID 4377374) was expressed <SEQ ID 281; cp7374>:

```

10      1 MDKQSSGNSG CIWHPFTQSA LDSTPIKIVR GEGAYLYAES GTRYLDAISS
      51 WWCNLHGHGH PYITKKLCEQ AQKLEHVIFA NPTHEPALEL VSKLAPLLPE
      101 GLERFFFS DN GSTSIBIAMK IAVQYYNQN KAKSHFVGLS NAYHGDTFGA
      151 MSIAGTSPTT VPFHDLFLPS STIAAPYYGK ERLAIAQAKT VFSSENIAAF
      201 IYEPLLQAG GMLMYNPEGL KEILKLAKHY GVLCAIDEIL TGFGRGTGLF
      251 ASEFTDIPPD IICLSKGLTG GYLPLALTVT TKBIHDAFVS QDRMKALLHG
15      301 HTFTGNPLGC SAALASLDLT LSPECLQQRQ MIERCHQKQFQ EAHGSLWQRC
      351 EVLGTVLALD YPARATGYFS QYRDHLNRFF LERGVLLRPL GNTLYVLPFY
      401 CIQEEDLRIT YSHLQDALCL QPQ*
```

The cp7374 nucleotide sequence <SEQ ID 282> is:

```

20      1 ATGGACAAGC AATCATCAGG GAATTCAGGG TGTATCTGGC ACCCCTTCAC
      51 TCAATCTGCA TTAGATTCTA CACCCATAAA GATTGTAAGG GGAGAAGGTG
      101 CTTACCTCTA TCGCGAATCA GGAACAAGAT ATCTTGATGC GATATCTTCA
      151 TGGTGGTGCA ACCTCCACGG TCATGGGCAT CCCTACATTA CAAAAAATT
      201 ATGTGAGCAA GCACAGAAGT TAGAACATGT GATCTTCGCA AATTTCACCC
      251 ATGAACCGGC TCTAGAGCTC GTATCGAAAC TCGCTCCCTT CCTTCCTGAA
25      301 GGTCTAGAAC GTTCTTTTCT CTCTGACAAC GGATCAACGT CTATCGAAAT
      351 AGCAATGAAA ATTGCTGTGC AATATTACTA CAATCAAAAC AAGGCTAAGA
      401 GCCATTTTGT TGGACTCAGC AATGCCTATC ACGGAGATAC ATTTGGAGCT
      451 ATGTCGATAG CTGGCAGCAG CCCTACTACA GTTCCCTTTC ATGATCTTTT
      501 TCCTTCTTCC AGTACAATTG CTGCTCCCTA TTATGGCAAG GAAGAGCTTG
30      551 CCATTGCCCA AGCAAAAACA GTCTTTTCTG AAAGCAATAT CGCAGCGTTT
      601 ATCTATGAGC CGCTATTGCA AGGTGCTGGA GGGATGTTAA TGTATAATCC
      651 CGAAGGCCCTA AAGGAGATTG TCAAGCTTGC CAAGCATTAC GGGGTTCTCT
      701 GTATTGCTGA TGAATTTCTT ACTGGCTTTG GCCGTACGGG TCCACTGTTT
      751 GCTTCTGAAT TTACAGACAT TCCTCCTGAC ATTATCTGTC TTCTAAAGG
35      801 TCTTACAGGA GGCTATCTCC CTCTAGCCTT GACAGTAACC ACTAAAGAAA
      851 TTCATGATGC CTTTGTCTCC CAAGATCGGA TGAAGGCACT GCTTCATGGC
      901 CATACCTTCA CAGGAAATCC TTTAGGCTGT AGTGCTGCCC TCGCTTCTTT
      951 GGATCTCACC CTATCTCCAG AATGCCTACA ACAAAGGCAA ATGATAGAAC
40      1001 GGTGTCATCA AGAGTTTCAA GAAGCTCATG GTTCCCTATG GCAACGGTGT
      1051 GAGGTTCTGG GCACGGTACT CGCTCTAGAT TACCCTGCAG AAGCTACAGG
      1101 ATATTTTTC AATATAGAG ACCATCTCAA TCGCTTTTTC TTAGAACGTG
      1151 GAGTCTTCT TCGTCTTTTA GGAACACAC TGTATGTGCT GCCCCCTAC
      1201 TGTATCCAAG AAGAAGATCT CCGGATTATT TATTCTCACC TACAGGATGC
      1251 CCTATGTCTA CAACCACAGT AA
```

- 45 The PSORT algorithm predicts cytoplasm (0.2930).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 141A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 141B) and for FACS analysis.

- 50 These experiments show that cp7374 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 142

The following *C.pneumoniae* protein (PID 4377377) was expressed <SEQ ID 283; cp7377>:

```

1  MREETVSWSL EDIREIVHTP VFELIHKANA ILRSNFLHSE LQTCYLISIK
5  51  TGGCVEDCAY CAQSSRYHTH VTPEPMMKIV DVVERAKRAV ELGATRVCLG
101 AAWRNAKDDR YFDRVLAMVK SITDLGAEVC CALGMLSEEQ AKKLYDAGLY
151 AYNHNLDDSP EFYETIITTR SYEDRLNTLD VVNKSGISTC CGGIVGMGES
201 EEDRIKLLHV LATRDHIPES VPVNLWPID GTPLQDQPI SFWEVLRTIA
251 TARVVFPFSM VRLAAGRAFL TVEQQTLCLF AGANSIFYGD KLLTVENNDI
301 DEDAEMIKLL GLIPRPSFGI ERGNPCYANN S*

```

The cp7377 nucleotide sequence <SEQ ID 284> is:

```

1  ATGCGTGAAG AAACGTGATC CTGGTCATTA GAAGACATCC GCGAAATTTA
51 TCACACTCCC GTATTTGAGC TGATTCACAA AGCCAATGCC ATATTGCGTA
101 GTAATTTCCCT CCATTTCAGAA CTGCAGACTT GCTATCTGAT TTTCGATTAAA
151 ACTGGTGGAT GCGTTGAAGA TGGCGCTAC TGTGCCCAAT CTTCCCGCTA
15  201 TCATACCCAC GTCACACCAG AACCTATGAT GAAAATGTGA GACGTTGTGG
251 AAAGGGCAAA ACGTGTCTGA GAGCTAGGCG CCACTCGTGT GTGTCTTGGG
301 GCTGCTTGGC GCAATGTCAA GGACGATCGA TACTTTGATA GAGTCCCTCGC
351 TATGGTGAAA AGTATCACAG ATCTCGGAGC CGAGGTTTGT TGTGCTTTAG
401 GCATGCTCTC CGAAGAGCAA GCTAAAAAAC TGTATGATGC AGGACTTTAT
20  451 GCCTACAATC ATAATTTAGA CTCTTCTCCG GAATTCATG AAATAATAAT
501 CACAACACGT TCTTATGAAG ATCGCCTCAA CACTCTGAT GTAGTAAATA
551 AATCTGGCAT TAGTACATGC TGGCGTGGTA TTGTAGGTAT GGGAGAATCT
601 GAAGAAGACC GTATAAAGCT TCTTCATGTT CTGCAACAA GAGATCATAT
25  651 CCCAGAATCC GTACCTGTAA ATTTACTTTG GCCGATTGAC GGCACGCCTT
701 TGCAAGACCA GCCTCCGATT TCTTCTGTTG AAGTCTGCG AACCATAGCA
751 ACGGCACGGG TTGTTTTCCT CAGATCCATG GTACGACTTG CTGCAGGACG
801 CGCTTTCTCT ACAGTAGAAC AACAAACCTT ATGTTTCTA GCCGGTGCCA
851 ACTCCATATT CTATGGAGAT AAACGTGTTG CTGTAGAAAA CAATGATATA
901 GATGAAGATG CTGAAATGAT CAAACTTTTA GGCTTAATCC CTCGCCCTTC
30  951 ATTTGGAATA GAAAGAGGTA ACCCATGTTA TGCCAACAAT TCCTAA

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The PSORT algorithm predicts cytoplasm (0.2926).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 142A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 142B) and for FACS analysis.

These experiments show that cp7377 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 143

The following *C.pneumoniae* protein (PID 4377407) was expressed <SEQ ID 285; cp7407>:

```

1  MVEPNNSWFR MCGNFNCEWV EVTTTEETTR QSASDISEEA GSSGGAAPIT
40  51  TQPTKITKVE KRVQFNTAQG DESTIHIQE AGELVDSILS HRRTQGCTEY
101 CYDSYATGCG QRCGSFGRLI CGTYKACCLD REDNQVAGLV HECEQTHGPI
151 AVALAAKTMG LNLMLVEKN TILSEEQKNE FRQHCSEAKT QLYGTMQSLS
201 QNFFLEGVNS IRERGLDDSL VQAVLSFIAT RSWERTIESE EASGTSSASN
251 STRIPACYIL NTSPLTTSRL SCGSRDARRP SSVGAEPQYV AKKYNDNGMA
45  301 RQLGKIQVTN LKTGDFSALG PFGLLIVKML NSFLLSASQS TSSILKHTGG
351 EICYTCPNFR DIVVLLMLAI GYCPANTDET SVVDIHMIDD PIMTIFYRLQ
401 YSYRTGRTSA SFLKKKPSLV RQESLDCPTP AESVPLMSSL EEDENEDDD
451 EDGNLAYQQR ILECSGHLQT LFLGKINKE *

```

The cp7407 nucleotide sequence <SEQ ID 286> is:

```

1  ATGTTTGGCC CAAATAATTC TTGGTTCAGA ATGTGTGGAA ATTTCAACTG
50  51  CGAATGGGTT GAAGTAACAA CAACAGAAGA AACACGCGG CAATCGGCTT
101 CAGATATAAG CGAAGAAGCT GGTTCGAGTG GAGGAGCTGC TCCTATAACT
151 ACGCAACCTA CTAAATTTAC AAAAGTAGAG AACGTGTCC AATTTAATAC

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201 TGCTCAAGGT GATGAAAGTA CAATACACAT GATCCAAGAA GCAGGAGAAT
 251 TGGTAGACTC CATTCATCA CATAGACGAA CGCAAGGATG TACAGAGTAT
 301 TGTATGACA GTTACGCAAC TGGATGTGGT CAGCGTTGCG GATCTTTTGG
 351 AAGACTCAT TGTGGAACGT ATAAAGCGTG TTGCTTAGAC AGAGAGGATA
 401 ATCAGGTTGC TGGACTTGTC CATGAATGCG AACAGACCCA TGGTCCTATT
 451 GCCGTTGCTT TAGCTGCTAA AACTATGGGC CTCAACTTAA TGGAACTTGT
 501 AGAAAAAAC ACTATTTTGT CTGAAGAACA GAAAAATGAA TTTAGACAGC
 551 ATTGCTCGGA AGCTAAAACC CAACTCTATG GAACGATGCA GAGCCTTTCT
 601 CAAAACCTTT TCCTTGAAGG AGTCAACAGC ATTAGAGAAC GCGGTCTAGA
 651 CGATTCACATA GTCCAAGCCG TGCTAAGCTT TATTGCTACA AGGTCTTGGG
 701 AAAAACTAT AGAATCAGAG GAAGCCTCAG GAACATCTTC TGCTTCTAAT
 751 TCTACACGCA TTCCTGCGTG CTATATCTTA AATACGAGCC CCTTAACGAC
 801 GTCACGCCA TCCTGTGGAT CAAGAGATGC GCGACGCCCA TCTTCAGTCG
 851 GTGCAGAGCC CCAGTACGTA GCAAAAAAAT ACAATGACAA TGGCATGGCC
 901 AGACAATTAG GAAAAATCCA AGTCACCAAT CTAAAAACAG GAGATTTTTT
 951 AGCTTTAGGT CCTTTTGGTC TCCTGATTGT GAAATGCTG AATAGCTTTC
 1001 TCTTATCTGC ATCACAAGC ACATCTTCTA TTCTAAAGCA CACAGGTGGA
 1051 GAAATATGTT ATACGTGCCC AAATTTTCGT GATATCGTCG TTTTATTGAT
 1101 GTTAGCGATT GGCTATTGCC CTGCAAATAC CGATGAGACA TCTGTCGTAG
 1151 ATATACACAT GATAGATGAT CCGATTATGA CCATCTTCTA TCGACTACAA
 1201 TACAGCTATA GAACAGGGAA AACTTCAGCA TCGTTTTTAA AAAAGAAACC
 1251 CTCATTAGTA AGACAGGAAA GTCATTGATTG TCCTACCCCT GCAGAATCTG
 1301 TCCTCTCAT GTCAAGTCTC GAAGAAGAAG ATGAAAATGA AGATGATGAT
 1351 GAGGATGGGA ATTTGGCGTA TCAACAGCGT ATCCTTGAAT GCTCGGGTCA
 1401 TTTACAACT CTATTTTCTAG GGATAAAAAT AAACAAAGAA TAA

The PSORT algorithm predicts inner membrane (0.1319).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 143A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 143B) and for FACS analysis.

30 These experiments show that cp7407 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone:

Example 144

The following *C.pneumoniae* protein (PID 4376432) was expressed <SEQ ID 287; cp6432>:

35 1 MTRSTIESSD SLCSRSFSQK LSVQTLKNLC ESRLMKITSL VIAFLTLIVG
 51 GALIALAGGG VLSFPLGLIL GSVLVLFSSI YLVSCCKFFT LKEMTMTCSV
 101 KSKINIWF EK QRNKDIEKAL ENPDLFGENK RNVGNRSARN QLEMILHETD
 151 GIILKRYMKG AKMYFYL*

The cp6432 nucleotide sequence <SEQ ID 288> is:

40 1 ATGACTAGAA GTACTATTGA AAGCAGTGAT TCGCTATGCT CAAGGTCTTT
 51 TTCTCAAAAA TTAAGTGTC AGACATTAAA AAATCTCTGT GAAAGTAGAT
 101 TAATGAAGAT CACTTCTCTT GTGATTGCTT TCCTAACTCT AATTGTGGGG
 151 GGTGCTCTTA TAGCTTTAGC AGGAGGGGGG GTTCTTTCTT TCCCTCTTGG
 201 GCTAATCTTA GGAAGCGTAC TCGTTTTGTT TTCCTCTATC TATTTAGTCT
 251 CTTGTTGTAA ATTTTCTACT TTAAAAGAGA TGACAATGAC CTGTAGTGTC
 45 301 AAATCTAAAA TCAATATATG GTTTGAAAAG CAACGAAACA AAGACATCGA
 351 AAAGGCATTA GAGAATCCAG ATCTCTTTGG AGAAAATAAG AGAAATGTTG
 401 GAAATCGTTC GGCAAGAAAT CAACTAGAAA TGATCTTACA CGAGACTGAC
 451 GGAATTATTT TGAAAAGATA TATGAAAGGA GCTAAAATGT ACTTTTATTT
 501 ATGA

50 The PSORT algorithm predicts inner membrane (0.5394).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 144A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 144B) and for FACS analysis.

These experiments show that cp6432 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 145

The following *C.pneumoniae* protein (PID 4376433) was expressed <SEQ ID 289; cp6433>:

```

5      1  MNWVPKTIDH VDPESIDIR KVVSCYKLIK ECQPEFRSLI SELLGVIRCG
      51  LRLLRKSKYQ EQARTVDEED APLFCLTRSY YQDGYLTPLR AGPRDLINHY
     101  IHLRRRENPK HFFSPKHPCY YARLAFNESV CVYRELFDIR RLTKMYVEGD
     151  YSKEQEKNLQ AILSFVKFLD EGKDFLIEHK DTDLIGRGFT DVFCT*

```

The cp6433 nucleotide sequence <SEQ ID 290> is:

```

10      1  ATGAATTGGG TTCCAAAAC AATAGACCAT GTAGATCCAG AATCAGAGAT
      51  AGATATACGT AAAGTCGTCT CCTGCTATAA GTTGATAAAA GAATGTCAAC
     101  CTGAATTTTCG ATCTCTTATA AGTGAATTAC TAGGAGTGAT TCGGTGTGGC
     151  TTAAGACTAT TAAACGTTTC TAAGTATCAA GAACAGGCTA GAACTGTATC
     201  TGATGAAGAT GCACCTCTTT TCTGCCTGAC TCGTTCTTAT TATCAAGATG
15      251  GTTATCTCAC GCCATTAGA GCAGGACCTC GTGATCTTAT AAATCACTAT
      301  ATACACTTGC GTCGCCGAGA GAATCCTAAG CATTTTTCAT GTCTTAAGCA
     351  TCCATGTTAT TATGCTCGAT TGGCTTTTAA TGAGTCAGTG TGTGTCTATA
     401  GAGAACTCTT TGATATAGAG CGACTTACAA AAATGTATGT CGAGGGTGAT
     451  TATTCTAAAG AACAAAGAGAA AAACCTACAG GCTATCTTAA GTTTTGTGAA
20      501  AACTCTAGAT GAAGGAAAGG ACTTTCTTAT TGAACATAAA GATACCGATC
     551  TCAATTGGGAG AGGTTTACT GATGTGTTCT GCACTTAA

```

The PSORT algorithm predicts cytoplasm (0.4068).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 145A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 145B) and for FACS analysis.

These experiments show that cp6433 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 146

The following *C.pneumoniae* protein (PID 4376643) was expressed <SEQ ID 291; cp6643>:

```

30      1  MGYLPVSATD VLFESPAAPL INSANTQNRK LIELEKQQA ESSPRITTSV
      51  ILEVLLVIGC CLIVLSLLAI RPALQFTLET GHPAAIAVLA VSGTILLVAV
     101  IILFCFLAAV PFAARKTYKY VRTVDDYASW HSHQQTPTLG TIFSGIVYAE
     151  SQAQL*

```

The cp6643 nucleotide sequence <SEQ ID 292> is:

```

35      1  ATGGGATATC TTCCAGTATC TGCTACGGAC GTTCTTTTTC AAAGTCCAGC
      51  CGCTCCCTTA ATCAATAGCG CAAACACACA AAATCAGAAA CTCATAGAAC
     101  TCAAGGGGAA GCAGCAAGCT GAGTCTTCTC CACGGACAAT CACTTCTGTC
     151  ATATTGGAAG TTCTCCTAGT GATCGGATGC TGCCTCATAG TTCTTAGTTT
     201  ATTGGCAATC CGCCCTGCTC TGCAATTCAC TCTAGAACT GGACATCCAG
40      251  CTGCCATTGC AGTCCTTGCT GTCTCAGGAA CAATTCTATT GGTGGCTGTT
      301  ATCATCTTGT TTTGCTTTCT AGCAGCTGTG CCATTCGCTG CTAAGAAAAC
     351  TTATAAATAT GTTAAGACGG TTGATGACTA TGCTTCTTGG CATTCTCATC
     401  AGCAAACACC GACCCTAGGC ACTATCTTTT CAGGTATCGT CTATGCAGAA
     451  TCCCAGGCGC AATTATAG

```

45 The PSORT algorithm predicts inner membrane (0.6859).

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The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 146A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 146B) and for FACS analysis.

These experiments show that cp6643 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 147

The following *C.pneumoniae* protein (PID 4376722) was expressed <SEQ ID 293; cp6722>:

```

1  VSSTLNGVFP SSLPEESADL FITNKEIVAL GEKGNVFLTH SIPMHIAAIT
51  ILVIVALAGI AIICLGCSYQ SILLIAVGIV LTILTLCLQ ALVGFIFIR
101 QLPQQLHTTV QFIREKIRPE SSLQLVTNAQ RKTTQDTLKL YEELCDLSQK
151 EFKLQSTLYQ KRFELSHKNE KTNQN*

```

The cp6722 nucleotide sequence <SEQ ID 294> is:

```

1  GTGTCTAGTA CTTTAAACGG GGTATTTCCC TCATCCCTTC CGGAAGAGTC
51  TGCTGATTTA TTCATTACGA ATAAGGAGAT CGTAGCTTTG GGGGAGAAGG
151 GCAATGTTTT TCTCACCCAC TCCATTCCCTA TGCATATTGC TCGGATTACG
201 ATCTTAGTGA TTGTAGCTCT TGCTGGAATC GCTATTATCT GTTTGGGTTG
251 CTATAGCCAA AGCATTCTGT TGATTGCCGT TGGCATGTGT CTTACTATTT
301 TGACTCTTCT CTGCCTACAA GCCTTGGTAG GATTTATTAA ATTCATCCGG
351 CAGCTCCCTC AGCAGCTCCA TACGACAGTA CAATTATCA GGGAGAAGAT
401 TCGACCTGAA TCCTCTCTAC AGCTTGTAAC CAATGCACAG AGAAAAACCA
451 CTCAAGATAC GCTAAAGTTA TACGAAGAAC TCTGCGACCT CTCACAAAAA
501 GAGTTCAAAC TGCAATCAAC TCTTTATCAA AAACGTTTTC AGCTTTCTCA
501 CAAGAATGAA AAGACAAATC AAAACTAG

```

The PSORT algorithm predicts inner membrane (0.6668).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 147A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 147B) and for FACS analysis.

These experiments show that cp6722 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 148

The following *C.pneumoniae* protein (PID 4377253) was expressed <SEQ ID 295; cp7253>:

```

1  MSELAPCSTG LQVPHTQVH HALDTRRVIL TIAACLSLIA GIVLVGLGAA
51  AILPSLPGVI GGMILILFSS IALIVLYKKT REVDQIALEP LPEMISKDQS
101 IIDFVKTRDY ASLEKKATFA YTHTHYYDGS MVFYREIPRF MLGSYLALRK
151 DMDRQALF*

```

The cp7253 nucleotide sequence <SEQ ID 296> is:

```

1  ATGAGCGAGC TCGCCCCCTG CTCGACAGGA TTGCAGATGG TCCCCCATA
51  GCAGGTCCAT CATGCCCTTG ATACGCGGAG AGTCATTCTA ACGATAGCCG
101 CCTGTCTGTC TTTAATTGCA GGAATCGTGT TGGTTGGCTT AGGTGCTGCA
151 GCAATCCTGC CCTCGCTTTT TGGAGTCATT GGAGGAATGA TTCTTATCT
201 GTTTTCTTCG ATCGCCCTCA TTTATTTATA CAAGAAGACA AGGGAGGTGG
251 ATCAGATTGC TCTGGAGCCT CTTCCCTGAGA TGATTCTTAA AGATCAAAGC
301 ATTATAGATT TTGTAAAGAC ACGAGACTAT GCATCTTTAG AAAAGAAAGC
351 GACCTTTGCT TATACTCATA CTCATTATTA CGATGGAAGC ATGGTCTTCT
401 ATAGGGAGAT CCTTAGATTT ATGTTAGGCT CTTATCTCGC GCTTCGCAA
451 GACATGGACC GCCAAGCTCT TTTTGA

```

The PSORT algorithm predicts inner membrane (0.5394).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 148A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 148B) and for FACS analysis.

These experiments show that cp7253 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 149

The following *C.pneumoniae* protein (PID 4376264) was expressed <SEQ ID 297; cp6264>:

```

1  VISGLLFLLV RREVPVRSR EIPRGVSVTP SEEPALAKAQ KEPETKKILD
51  RLPKELDQLD TYIQEVFACL ERLKDPKYED RGLLTEAKRK LRVPDVVEKD
101 MMSEFLDIQR VLNERAYYVE HCQDPLENIA YEIFSSQELR DYVCAGVCGY
151 LPSGDARADR LKRSVKVMD RFRVTVKSW EASVMLDHSY GVARELFKKA
201 VGVLEESVYK ILFKSYRDAF YCEKAKIQR DGRFKWL*

```

The cp6264 nucleotide sequence <SEQ ID 298> is:

```

1  GTGATTTCCG GACTTCTATT CCTTCTAGTA AGACGAGAGG TTCCGACAGT
51  ACGTTCAGAG GAAATTCCCA GAGGGGTTTC TGTGACCCCT TCTGAAGAGC
101 CTGCTCTAGA GAAGGCTCAA AAAGAACCGG AGACAAAGAA AATTTTAGAT
151 CGGTGCCCGA AGGAATTGGA TCAGTTAGAT ACGTATATTC AGGAAGTGTT
201 TGCAATGTTA GAGAGGCTGA AGGATCCTAA GTACGAAGAT CGAGGTCCTT
251 TAACAGAGGC GAAGGAGAAA CTTTCGAGTTT TTGACGTTGT TGAGAAAGAT
301 ATGATGTCAG AGTTTCTAGA CATACAACGA GTGTTGAATG AGGAAGCATA
351 TTATGTAGAA CATTGTCAAG ATCCCCTAGA GAATATAGCC TACGAGATTT
401 TCTCTTCCCA AGAGCTTCGT GATTACTACT GTGCAGGGGT GTGTGGGTAT
451 TTGCCTTCTG GGGATGCTCG AGCGGATCGA TTAAGAGAGT CAGTTAAGGA
501 GGTAATGGAT CGCTTTATGA GGGTGACCTG GAAATCTTGG GAGGCATCAG
551 TCATGTTTGA TCATAGCTAT GGGGTAGCGC GAGAGTTATT CAAGAAGGCA
601 GTAGGAGTAC TAGAGGAGAG TGTCTATAAA ATTCTGTTTA AGAGCTATAG
651 AGATGCGTTT TATGAATGTG AGAAGGCAAA GATCCAGAGG GATGGGCGTT
701 TCAAATGGTT ATAG

```

The PSORT algorithm predicts cytoplasm (0.2817).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 149A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 149B) and for FACS analysis.

These experiments show that cp6264 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 150

The following *C.pneumoniae* protein (PID 4376266) was expressed <SEQ ID 299; cp6266>:

```

1  MLLISGALF LTLGIPGLSA AISFGLGIGL SALGGVLMIS GLLCLLVKRE
51  IPTVRPEEIP EGVSLAPSEE PALQAAQKTL AQLPKELDQL DTDIQEVFAC
101 LRKLKDSKYE SRSFLNDAKK ELRVDFVVE DTLSEIFELR QIVAQEGWDL
151 NFLINGRSL MMTAESLSD LFHVSKRLGY LPSGDVRGEG LKKSAREIVA
201 RLMSLHCEIH KVAVAFDRNS YAMAEKAFK ALGALESYV RSLTQSYRDK
251 FLESEKAKIP WNGHITWLRD DAKSGCAEKK LGMPRNVGRN LGKQSFQ*

```

The cp6266 nucleotide sequence <SEQ ID 300> is:

```

1  ATGCTCTTAC TGATTTCAGG AGCTCTCTTT CTGACGTTAG GGATTCAGG
51  ATTGAGTGCA GCAATTTCTT TTGGATTAGG CATCGGTCTC TCCGCATTAG
101 GAGGAGTGCT GATGATTTTC GGAATCTAT GTCTTTTAGT AAAACGAGAG
151 ATTCCGACAG TACGACCAGA AGAAATTCCT GAAGGGGTTT CGCTGGCTCC

```

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201 TTCTGAGGAG CCAGCTCTAC AGGCAGCTCA GAAGACTTTA GCTCAGCTGC
 251 CTAAGGAATT GGATCAGTTA GATACAGATA TTCAGGAAGT GTTCGCATGT
 301 TTAAGAAAGC TGAAAGATTC TAAGTATGAA AGTCGAAAGT TTTTAAACGA
 351 TGCTAAGAAG GAGCTTCGAG TTTTGTGACTT TGTGGTTGAG GATACCCTCT
 5 401 CCGAGATTTT CGAGTTGCGG CAGATTGTGG CTCAGAGAGG ATGGGATTTA
 451 AACTTTTGA TCAATGGGGG ACGAAGCCTC ATGATGACTG CAGAATCTGA
 501 ATCGCTTGAT TTGTTTCATG TATCGAAGCG GCTAGGGTAT TTACCTTCTG
 551 GGGATGTTTC AGGGGAGGGG TTAAGAAAT CTGCCAAGGA GATAGTCGCT
 601 CGTTTGATGA GCTTGCATTG CGAGATTCAC AAGGTGGCGG TAGCGTTTGA
 10 651 TAGGAATTCC TATGCGATGG CAGAAAAGGC GTTTGCGAAA GCGTTGGGAG
 701 CTTTAGAAGA GAGTGTGTAT CGGAGTCTGA CGCAGAGTTA TAGAGATAAA
 751 TTTTGGGAGA GCGAGAGGGC GAAGATCCCA TGGAATGGGC ATATAACCTG
 801 GTTAAGAGAT GATGCGAAGA GTGGGTGTGC TGAAAGAAG CTCGGGATGC
 851 CGAGGAACGT TGAAGAAAT TTAGGAAAG AGTCTTTTGG GTAG

15 The PSORT algorithm predicts inner membrane (0.3590).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 150A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 150) and for FACS analysis.

20 These experiments show that cp6266 is a surface-exposed and immunoaccessible protein and that they it is a useful immunogen. These properties are not evident from the sequence alone.

Example 151

The following *C.pneumoniae* protein (PID 4376895) was expressed <SEQ ID 301; cp6895>:

1 MKIKKSFQYS LCQAKRFQNM LPNHFDPCIQ PVNLQLKQDR LAYGELIILL
 25 51 SKYQQKTFSS LLKEETCSLN RAKQHLLYKI LRDFNTMQHL RSLGLNGWGE
 101 IPMSPL*

The cp6895 nucleotide sequence <SEQ ID 302> is:

1 ATGAAGATTA AAAAATCTTT TCAATACAGT TTATGCCAAG CAAAGAGATT
 51 TCAGAACATG CTGCCAAACC ACTTTGATCC ATGTTTGCAG CCAGTGAATT
 101 TACAACTCAA ACAAGACAGA TTGGCATAAG GGGAGCTCAT CATATTGCTA
 30 151 TCTAAATATC AACAAAAGAC CTTTTCCTCT TTGTTGAAGG AAGAAACATG
 201 TTCTCTTAAT CGTGCGAAGC AGCACTTATT GTATAAGATT TTGAGAGATT
 251 TTAATACTAT GCAGCATCTA AGGTCCCTCG GATTAAATGG TTGGGGAGAG
 301 ATCCCTATGA GTCCTGCCT CTAA

The PSORT algorithm predicts cytoplasm (0.3264).

35 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 151A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 151B) and for FACS analysis.

These experiments show that cp6895 is a surface-exposed and immunoaccessible protein and that it is a useful immunogen. These properties are not evident from the sequence alone.

40 Example 152 and Example 153

The following *C.pneumoniae* protein (PID 4376282) was expressed <SEQ ID 303; cp6282>:

1 MSLLNLPSQ DSASEDSTS SQIFDPIRNR ELVSTPEEKV RQRLLSFLMH
 45 51 KLNYPKLLII IEKELKTLFP LLMRKGTLP KRRPDILIT PPTYTDAQGN
 101 THNLGDPKPL LLIECKALAV NQNALQQLS YNYSIGATCI AMAGKHSQVS
 151 ALFNPKTQTL DFYPGLPEYS QLLNYFISLN L*

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The cp6282 nucleotide sequence <SEQ ID 304> is:

```

1  ATGTCCTTAT TGAACCTTCC CTCAGCCAG GATTCTGCAT CTGAGGACTC
51  CACATCGCAA TCTCAAATCT TCGATCCCAT TAGAAATCGG GAGTTAGTTT
101 CTACTCCCGA AGAAAAAGTC CGCCAAAGGT TGCTCTCCTT CCTAATGCAT
151 AAGCTGAAC TACCCTAAGAA ACTCATCATC ATAGAAAAAG AACTCAAAAC
201 TCTTTTTCCT CTGCTTATGC GTAAAGGAAC CCTAATCCCA AAACGCCGCC
251 CAGATATTCT CATCATCACT CCCCCACAT ACACAGACGC ACAGGGAAAC
301 ACTCACAACC TAGGCGACCC AAAACCCCTG CTAATTATCG AATGTAAGGC
351 CTTAGCCGTA AACCAAAATG CACTCAAACA ACTCCTTAGC TATAACTACT
10  401 CTATCGGAGC CACCTGCATT GCTATGGCAG GGAACACTC TCAAGTGTCA
451 GCTCTCTTCA ATCCAAAAC ACAAACTCTT GATTTTATC CTGGCCTCCC
501 AGAGTATTCC CAACTCCTAA ACTACTTTAT TTCTTTAAAC TTATAG

```

The PSORT algorithm predicts cytoplasm (0.362).

The following *C.pneumoniae* protein (PID 4377373) was also expressed <SEQ ID 305; cp7373>:

```

15  1  MSTPTVKHFI HTASRWEFVL KEIVASNYWH AQWINTLSFL ENSGAKRISA
51  SEHPTFVKKE VLKHAAREFR HGHYLTQIS RISETSLPDY TSKNLLGGLL
101 TKYLYLHLLDL RTCRVLENEY SLGQTLKTA AYILVTYAIE LRASELYPLY
151 HDILKEAQSK ITVRSIILEE QGHLQEMERE LKDLPHGEEL LGYACQFEGE
201 LCLQFVERLE QMIFDPSSTF TKF*

```

20 The cp7373 nucleotide sequence <SEQ ID 306> is:

```

1  ATGCTACAA CCACAGTAAA ACACTTTATC CACACAGCCT CTCGTTGGGA
51  GCCCGTTCTC AAAGAGATCG TAGCTTCCAA CTATTGGCAT GCACATGGA
101 TAAATACCC TCCCTTTTTA GAAATAGTG GAGCAAAAAA AATCTCCGCA
151 AGTGAACATC CTACGGAGGT AAAGGAAGAA GTTTTAAAC ATGCTGCTGA
25  201 AGAATTTCTG CATGGTCACT ATCTAAAAAC TCAGATTTCT AGAATCTCAG
251 AGACTTCTCT CCTTGACTAT ACATCTAAAA ATCTTCTGGG AGGCTTACTT
301 ACAAAATAT TACCTCCATCT TCTAGATTTA AGGACGTGCC GAGTACTGGA
351 AAATGAATAC TCCCTATCGG GACAAACGTT AAAAACTGCA GCGTATATTT
401 TAGTTACCTA CGCAATCGAA CTTCTGCTTT CTGAACTTTA TCCTCTGTAT
30  451 CACGATATTC TGAAAGAAGC TCAAAGTAAA ATAACGGTAA AATCCATTAT
501 CTTAGAAGAG CAAGGCCATC TGCAAGAGAT GGAACGTGAA CTTAAAGATC
551 TCCCCACGGG GGAGGAACTC TTAGGCTATG CTTGCCAATT CGAAGGGGAG
601 CTTTGCTTGC AGTTTGTAGA GAGATTAGAA CAAATGATCT TCGATCCTTC
651 CTCGACTTTT ACAAGTTCT AG

```

35 The PSORT algorithm predicts cytoplasm (0.1069).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 152A; 6282 = lanes 8 & 9; 7373 = lanes 2-4). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 152B & 153) and for FACS analysis.

40 These experiments show that cp6282 & cp7373 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

Example 154 ,
 Example 155 ,
 Example 156 ,
 Example 157 and
 45 Example 158

The following *C.pneumoniae* protein (PID 4376412) was expressed <SEQ ID 307; cp6412>:

```

1  MSSSEVVFQT VHGLGFGGLS SKSVVPFKKS LSDAPRVVCS ILVLTGLGA
51  LVCGLAITCW CVPGVILMGG ICAIVLGAIS LALSFLWLWG LFSNCCGSKR
101 VLPGEGLLRD KLLDGGFSRA APSGMGLPGD GSPRASTPSC LEELQARIQA
50  151 VTQAIDQMSD D*

```

The cp6412 nucleotide sequence <SEQ ID 308> is:

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1 ATGAGCAGTT CGGAAGTTGT TTTCCAGACA GTTCATGGCC TTGGCTTTGG
 51 TGGATTGTCT TCAAAAAGTG TTGTCCCTTT TAAGAAAAGT CTTTCGGATG
 101 CGCCCCGTGT TGTGTGCTCG ATTTTAGTTT TGACTCTGGG GTTGGGAGCG
 151 CTTGTTTGTG GTATTGCCAT TACTTGTGG TGTGTCCCGG GAGTTATTTT
 5 201 AATGGGGGGA ATTTGCGCTA TAGTTTtagg TGCAATTTCT TTAGCTTTAA
 251 GTCTATTTTG GTTGTGGGGT TTATTTTCTA ATTGTGTGG TTCTAAGAGA
 301 GTTTTACCGG GTGAGGGATT GCTACGGGAT AAGCTTTTAG ATGGTGGATT
 351 TTCAAGAGCG GCACCTTCAG GAATGGGACT TCCGGGTGAT GGATCTCCAA
 401 GAGCGTCAAC GCCATCTTGC CTAGAGGAAC TTCAAGCAGA GATACAGGCA
 10 451 GTTACTCAAG CTATCGATCA GATGTCAGAT GATTGA

The PSORT algorithm predicts inner membrane (0.4864).

The following *C.pneumoniae* protein (PID 4376431) was also expressed <SEQ ID 309; cp6431>:

1 LLAGGSLVTT YPKQGRLRS PEQLRVLDL VQSYNHLHA IELDCGAIPQ
 51 DLIGATYIIT FADPSTYILS LRSYQANSPS DDTWGIWFGS IDDPVQAVIS
 15 101 FLKDHFALP STLQDPLLC TNK*

The cp6431 nucleotide sequence <SEQ ID 310> is:

1 TTGCGAGCAG GAGGTAGTCT TGTTACAACA TACCCTAAGG AAGGTCAGAG
 51 ATTGCGCTCC CCAGAACAGT TAAGAGTTCT GGATGATTTA GTGCAAAGCT
 101 ATCCAAATCA CCTACATGCG ATTGAACCTG ATTGTGGTGC AATCCCTCAA
 20 151 GATTTGATCG GAGCCACCTA TATCATCAGG TTCGCGGATT TTCCACCTA
 201 TATCTCTCT TTAAGAAGCT ACCAAGCCAA TTCTCCCTCC GATGATACAT
 251 GGGGGATTG GTTTGATCT ATTGACGATC CTGTTCAAGC AGTCATATCA
 301 TTTTAAAAG ATCATGGATT TGCTCTTCCC TCGACCTTAG CTCAAGATCC
 351 TTTGCTTTGT ACTAACAAGT AA

25 The PSORT algorithm predicts cytoplasm (0.2115).

The following *C.pneumoniae* protein (PID 4376443) was also expressed <SEQ ID 311; cp6443>:

1 MIMTTISNSP SPALNPELSL IPPPTLVSSG TQTSLAYTIP AQGRRSTLRI
 51 ILDIFIILG LATIISTFIV IFFLNGLNLL STPSIISSSC LIIVGLLFLI
 30 101 MGLYFMISL DQGLVGLLQK ELSQAEBREE EYIQEIEALR GAPRAESPT
 151 SPSTWL*

The cp6443 nucleotide sequence <SEQ ID 312> is:

1 ATGATTATGA CTACTATATC TAACTCACCC TCCCCTGCAT TGAATCCCGA
 51 ACTTTCCTT ATTCTCCAC CAACACTTGT ATCTTCAGGT ACGCAAACAT
 101 CTCTAGCTTA TACGATCCCC GCACAAGGAC GAAGATCCAC CCTACGTATT
 35 151 ATATTAGATA TATTCTATTAT CATCTTTGGT TTAGCTACGA TCATTTCTAC
 201 CTTTATTTGTT ATTTCTTTT TAAATGGGCT GAACCTGCTC TCGACCCCAT
 251 CTATTATCTC TTCGTCATGT TTAATCATTG TTGGATTGCT TTTTGTGATT
 301 ATGGGGTTAT ATTTCTATGAT CTCGAGTTTG GATCAGGGGC TTGTAGCCCT
 351 TCTGCAAAAG GAACTCTCTC AAGCCGAAGA AAGAGAAGAA GAGTATATCC
 40 401 AGGAAATCGA AGCTTTAAGA GGAGCTCCTA GAGCAGAATC TCCACAGAG
 451 TCTCCTAGTA CCTGGTTATG A

The PSORT algorithm predicts inner membrane (0.5585).

The following *C.pneumoniae* protein (PID 4376496) was also expressed <SEQ ID 313; cp6496>:

1 MLIGRYSSDD QFTEATKNTF TIILGFVRD NLEGLTNPIS EIVSETSSSI
 45 51 KDSVLRSLPI LGSILGCARL YSTLSTNDPL DETQEKIWHI IFGALETGLL
 101 GILILLFKII FVILHCIFHL VIGFCK*

The cp6496 nucleotide sequence <SEQ ID 314> is:

1 ATGCTAATAG GCAGATACAG TAGTGATGAC CAATTCAGT AAGCAACAAA
 50 51 AAACACCCCA ACCATAATTA AGCTAGGTTT TGTAGAGAT AATCTCGAGG
 101 GATTAACGAA CCCTATCTCT GAAATCGTCT CGGAAACCTC CTCTCTATT
 151 AAAGATTCCG TTCTTCGCTC TCTTCTATT TTAGGTPCCA TTTTAGGATG
 201 CGCCCGACTT TACAGCACAC TCTCTACAAA TGATCTCTT GACGAACTC
 251 AAGAAAAGAT TTGGCACACT ATATTGGAG CCTTAGAAAC CTTAGGCTTA
 301 GGGATTCTCA TCCTCTTATT TAAAATTATT TTTGTTATAT TAACTGCAT
 55 351 ATTTTCATCTA GTTATTGGGT TCTGCAATA A

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The PSORT algorithm predicts inner membrane (0.5989).

The following *C.pneumoniae* protein (PID 4376654) was also expressed <SEQ ID 315; cp6654>:

```

1  MKTKMNSRKK AGQWAIFFNSP TPGVSSTLVL AWTPWGYDYK DVQDILERRK
51 PMSSSLSEKD SKEFLKNLFFV DLENGFTSV HIHAEBAFTP LDHTGKPHFK
101 RDNVYLPGLK LGALNEAAVQ ANVSADTQFT LFLTDQDECNP FHDKKRG*

```

The cp6654 nucleotide sequence <SEQ ID 316> is:

```

1  ATGAAACTA AAATGAAC TC TAGAAAAAAA GCAGGTCAAT GGGCAATTTT
51 CAATTCCTCA ACTCCTGGTG TCAGTTC AAC TTTAGTTTGA GCATGGACTC
101 CTTGGGGTTA TTACGACAAG GATGTACAAG ATATCTTAGA AAGAAAAGAT
151 CCGATGAGCT CTTGCGCTTTC TGAAAAAGAC TCAAAGGAGT TCTTGAAAAA
201 TCTGTTTGTG GATCTCTTAG AAAATGGCTT CACATCAGTA CATATTCACG
251 CAGAAGAAGC TTTCACCTCT CTTGATCATA CCGGGAAACC TCACCTTAAA
301 AGAGACAATG TGTACTTACC CGGAAAGTTG TTAGGCGCCT TGAATGAGGC
351 TGCAGGTACAA GCCAATGTAA GTGCGGATAC TCAATTTACA TTGTCCTTA
15 401 CTCAAGATGA GTGCAATCCT TTTCATGATA AGAAAAGAGG TTAA

```

The PSORT algorithm predicts cytoplasm (0.0730).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 154A; 6412 = lanes 2-3; 6431 = lanes 11-12; 6443 = lanes 5-6; 6496 = lanes 8-9; 6654 = lane 10; markers in lanes 1, 4, 7). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 154B, 155, 156, 157 & 158) and for FACS analysis.

These experiments show that cp6412, cp6431, cp6443, cp6496 & cp6654 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from their sequences alone.

Example 159 and Example 160

The following *C.pneumoniae* protein (PID 4376477) was expressed <SEQ ID 317; cp6477>:

```

1  LLKFFLVCEB LCILTVA THR ALLETPLALS FFKELKTRYV YRAKDILQLH
51 NYKGFITLNT SPLCS*

```

The cp6477 nucleotide sequence <SEQ ID 318> is:

```

30 1  TTGCTAAAGT TCTTTCTAGT ATGTGAAGAG TTATGTATAC TTA CTGTTGC
51 TACACATAGA GCTCTCTTAG AAACCTCTTT AGCTCTATCA TTTTPTAAAG
101 AACTTAAGAC AAAATATGTC TACAGGGCGA AAGACATACT ACAACTACAT
151 AACTATAAAG GATTTACTAT CCTTAATACA TCACCGTTAT GTTCTTAA

```

The PSORT algorithm predicts inner membrane (0.128).

The following *C.pneumoniae* protein (PID 4376435) was also expressed <SEQ ID 319; cp6435>:

```

1  LWSHFPRGFF MLPFCPTILL AKPFLNSEN Y GLERLAATVD SYFDLGQSQI
51 VFLSKQDQGI TVEELSAKDR KFKPGSMNCT LYTEDPILPA HNSFNCSDI
101 QMRTPISPIH *

```

The cp6435 nucleotide sequence <SEQ ID 320> is:

```

40 1  TTGTGGTCGC ATTTCCCAAG AGGATTTTTT ATGCTCCCTT TTGCGCTAC
51 CATCCTTCTT GCTAAACCTT TTTTAAATAG CGAGAATTAC GGCTTAGAAC
101 GTTTAGCTGC AACCGTAGAT TCTTATTTTG ATCTGGGACA GTCTCAAATA
151 GTCCTTCTTA GCAAACAGGA TCAAGGAATC ACTGTGGAAG AATTGAGTGC
201 TAAAGATAGG AAATTCAAGC CAGGCTCTAT GAACTGTACA CTGTACACTG
45 251 AAGATCCTAT CTTACCTGCT CATAATTCCT TTAGTAATTG CTCTGATATT
301 CAAATGCGTA CTCCGATTAG CCCTATACAT TAA

```

The PSORT algorithm predicts periplasmic space (0.4044).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 159A; 6435 = lanes 2-4; 6477 = lanes 5-7). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 159B & 160) and for FACS analysis.

- 5 These experiments show that cp6477 & cp6435 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequences alone.

**Example 161 and
Example 162 and
Example 163**

- 10 The following *C.pneumoniae* protein (PID 4376441) was expressed <SEQ ID 321; cp6441>:

```

1  VEAGANVLVI DTAHAHSGKV FQTVLEIKSQ FPQISLVVGN LVTAAEAASL
51  ABIGVDVAVK GIGPGSICIT RIVSGVGYPQ ITAITNVAKA LKNSAVTVIA
101 DGRIRYSGDV VKALAAGADC VMLGSLLAGT DEAPGDIVSI DEKLFKRYRG
151 MGS LGAMKQG SADRYFQTQG QKRLVPGGVE GLVAYKGSVH DVLYQILGGI
15  201 RSGMGYVGA E TLKDLKTKAS FVRITESGRA ESHIHNIYKV QPTLNY

```

The cp6441 nucleotide sequence <SEQ ID 322> is:

```

1  GTGGAAGCTG GAGCAAATGT TCTAGTCATT GACACAGCTC ATGCACACTC
51  TAAAGGAGTA TTCCAAACAG TTTTAGAAAT AAAATCCCAG TTCCACAAA
20  101 TTTCATTAGT TGTAGGGAAT CTTGTTACAG CTGAAGCCGC AGTTTCCTTA
151 GCTGAGATTG GAGTTGACGC TGTAAAGGTA GGTATGGCC CAGGATCTAT
201 CTGTACAAC T AGAATCGTTT CAGGGGTCGG TTATCCACAA ATTACTGCCA
251 TTACAAACGT AGCAAAAGCT CTTAAAAACT CTGCCGTGAC TGTAAATGCT
301 GATGGGAGAA TCCGCTATTC TGGAGATGTG GTAAAGCAT TAGCAGCAGG
351 AGCAGACTGT GTCATGCTAG GAAGTTTGCT TGCAGGGACT GATGAAGCTC
25  401 CTGGGGATAT CGTTTCTATC GATGAGAAGC TTTTAAAAG GTACCGCGGC
451 ATGGGATCTT TAGGCGCTAT GAAACAAGGA AGTGTGACC GGTATTTTCA
501 AACACAGGGA CAGAAAAGC TGGTTCTCTG GGGAGTTGAA GGACTAGTCG
551 CTTATAAAGG CTCGTGCCAC GATGTCTCT ATCAAATTTT AGGAGGAATA
601 CGCTCAGGTA TGGGGTATGT TGGAGCTGAA ACTCTCAAAG ATTTAAAAAC
30  651 TAAGGCTTCC TTTGTTCGAA TTAAGTAATC TGAAGAGCT GAAAGTCATA
701 TTCAATAATAT TTACAAAGTT CAACCAACCT TAAATATTA A

```

The PSORT algorithm predicts bacterial inner membrane (0.132).

The following *C.pneumoniae* protein (PID 4376748) was also expressed <SEQ ID 323; cp6748>:

```

1  LFSEGTALNL FRIFAPLRNR VTTEYSRARQ PDLHRIAIVY IGVLDSESSK
35  51 ILERLISYMS CIYSBSQMYL RFFMGKNVNO SAVLSKLHVE NLHIRCGFFS
101 EDAPPESEPF DLSIYVHTDR SCPLPTKKRS SSWELQTVEL PESIYPQSEF
151 LLMRPRMLS*

```

The cp6748 nucleotide sequence <SEQ ID 324> is:

```

1  TTGTTCTCTG AGGGGACAGC TCTAAATTTA TTTCGTATAT TTGCTCCACT
40  51 ACGCAACCGT GTGACTACAG AATACAGTCG TGCTAGGCAA CCCGACCTAC
101 ATAGAATTGC CATCGTCTAT ATAGGAGTTC TCGATTGAGA AAGTTCCAAG
151 ATCCTAGAGC GGCTAATCTC TTATATGAGT TGTATCTATT CTGAATCGCA
201 AATGTATTTA AGATTCTTTA TGGGCAAGAA TGTAAATCAA AGTGTGTGAC
251 TCTCAAAATT ACATGTAGAA AATCTGCACA TCCGTTGTGG GTTTTTCAGC
45  301 GAGGATGCTG TTCCAGAGAG TGAGCCCTTC GATCTCTCCA TCTACGTGCA
351 CACAGATCGT AGCTGTCTTC TCCCTACGAA AAAACGGAGC AGCTCCTGGG
401 AACTCCAAAC TGTAAGACTC CCAGAGTCAA TATATCCACA GTCGGAATTC
451 CTATTGATGA GACCTCGAAT GCTTTCGTAG

```

The PSORT algorithm predicts cytoplasm (0.170).

- 50 The following *C.pneumoniae* protein (PID 4376881) was also expressed <SEQ ID 325; cp6881>:

-174-

1 MRPHRKHVSS KSLALKQSAS THVEITTKAF RLSMPLKQLI LEKSDHLPPM
 51 ETIRVVLTSH KDKLGTEVHV VASHGKEILQ TKVHNANPYT AVINAFKKIR
 101 TMANKHSNKR KDRTKHDLGL AAKEERIAIQ EEQEDRLSNE WLPVEGLDAW
 151 DSLKTLGYVP ASAKKKISKK KMSIRMLSQD BAIRQLESAA ENFLIFLNEQ
 201 EHKIQCIVKK HDGNYVLIEP SLKPGFCI*

The cp6881 nucleotide sequence <SEQ ID 326> is:

1 ATGAGACCTC ATCGTAAACA CGTATCATCT AAAAGCTTAG CTTTAAAGCA
 51 ATCTGCATCA ACTCATGTAG AGATCACAAC AAAAGCCTTT CGTCTCTCTA
 101 TGCCTCTAAA ACAGCTGATC CTAGAGAAAA GCGACCACCT CCCCCCTATG
 151 GAAACAATCC GTGTGGTGCT AACCTCTCAT AAAGATAAGC TAGGCACCGA
 201 GGTGCATGTT GTAGCTTCTC ATGGCAAAGA AATCCTTCAA ACTAAGGTTT
 251 ATAACGCAAA CCCATACACT GCAGTGATCA ATGCTTTTAA GAAAATCCGC
 301 ACCATGGCAA ATAAGCACTC CAATAAACGT AAAGACAGGA CAAAACATGA
 351 TCTAGGTCTT GCAGCAAAAG AAGAACGTAT CGCAATACAG GAAGAACAAG
 401 AAGATCGCCT TAGCAACGAG TGGCTTCCTG TCGAAGGCCT CGATGCCTGG
 451 GATTCCTCTAA AAACCTCTTG GTATGTTCCC GCATCAGCGA AAAAGAAGAT
 501 CTCCAAGAAA AAGATGAGCA TTCGTATGCT ATCTCAAGAC GAGGCTATCC
 551 GCCAGCTAGA GTCTGCCGCA GAAAACTTCC TGATCTTCTT GAACGAGCAA
 601 GAGCATAAAA TCCAATGCAT TTATAAAAAA CATGACGGCA ACTATGTCTT
 651 TATTGAACCT TCCCTCAAGC CAGGATTCTG CATCTGA

The PSORT algorithm predicts cytoplasm (0.249).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 161A; 6441= lanes 7-9; 6748 = lanes 2-3; 6881 = lanes 4-6). The recombinant protein was used to immunise mice, whose sera were used in Western blots (Figures 161B, 162 & 163) and for FACS analysis.

These experiments show that cp6441, cp6748 & cp6881 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

Example 164 and Example 165 Example 166

The following *C.pneumoniae* protein (PID 4376444) was expressed <SEQ ID 327; cp6444>:

1 MEQPNCVIQD TTTVLYALNS FDPRLSDDTH RLKQSPLEA ENALGEFIEG
 51 LDTNSFPLEE VAIPILPGYH PKFYLSFIDR DDQGVHYEVL DGVFLKTVAA
 101 CIIENSFLTD SMSPELLSEV KEALKR*

The cp6444 nucleotide sequence <SEQ ID 328> is:

1 ATGGAGCAAC CCAATTGTGT GATTTCAGGAT ACTACAACCTG TTTTGTATGC
 51 CTTAAATAGC TTGTATCCTA GACTTAGTGA TGACACTCAC AGACTTGGGA
 101 AGCAATCACC TCTTGAAGCA GAAAATGCTC TTGGAGAATT TATTGAAGGT
 151 TTGGATACAA ATAGCTTTCC TTTAGAGGAA GTTGCCATTC CCATCCTGCC
 201 AGGTATATCAC CCTAAGTTTT ATTTATCTTT CATAGATAGG GACGATCAAG
 251 GTGTCCACTA TGAAGTTTTA GATGGCGTAT TTTTAAAGAC AGTCGCTGCT
 301 TGTATATATAG AGAACTCCTT CTTAACTGAT TCTATGAGCC CGGAGCTTCT
 351 CAGCGAAGTT AAGGAAGCTC TGAAACGATG A

The PSORT algorithm predicts cytoplasm (0.2031).

The following *C.pneumoniae* protein (PID 4376413) was also expressed <SEQ ID 329; cp6413>:

1 MAVQSIKEAV TSAATSVGCV NCSREAI PAF NTEERATSIA RSVIAAIIAV
 51 VAISLLGLGL VVLAGCCPLG MAAGATTMLL GVALLAWAIL ITLRLNLNIPK
 101 AEIPSPGNNG EPNERN SATP PLEGGVAGEA GRGGGSPLTQ LDLNSGAGS*

The cp6413 nucleotide sequence <SEQ ID 330> is:

1 ATGGCTGTTC AATCTATAAA AGAAGCCGTA ACATCAGCCG CAACATCAGT

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51 AGGATGTGTA AACTGTTCTA GAGAGGCTAT ACCAGCATTT AATACAGAGG
 101 AGAGAGCAAC GAGTATGCT AGATCTGTTA TAGCAGCTAT CATTGCTGTT
 151 GTAGCTATCT CCTTACTCGG ACTAGGTCTT GTAGTTCTTG CTGGTTGCTG
 201 TCCTTTAGGA ATGGCTGCGG GTGCTATAAC AATGCTGCTG GGTGTAGCAT
 251 TATTAGCTTG GGCAACTACTG ATTACTTTGA GACTGCTTAA TATACCTAAG
 301 GCTGAAATAC CGAGTCCAGG GAACAACGGT GAGCCTAATG AAAGAAATTC
 351 AGCAACTCCT CCTCTAGAGG GTGGTGTGTC AGGAGAAGCC GGTCCGCGCG
 401 GGGGTCACC TTTAACCCAA CTTGATCTCA ATTCAGGGGC GGAAGTTAG

The PSORT algorithm predicts inner membrane (0.6180).

10 The following *C.pneumoniae* protein (PID 4377391) was also expressed <SEQ ID 331; cp7391>:

1 MMLRVIELPL LPIKQALEKA FVQYNSYKAK LTKVEPCFRE SPAYITSEER
 51 LQSLDQTLER AYKEYQKRFG BPSRLESEVS GCREHLREQV KQFETQGLDL
 101 IKEELIFVSD VLFRKMVSCL VSTVHVPPME FYYEYFELHR LRLRAQWMAN
 151 ABIYSKVRKA FPEMLKETLE KAKAPREKEY WLLCEERKSK EKRLILNKIE
 201 AAQQRVKDLE PPPIKETGKQ KRKKEYSFFI RLKS*

The cp7391 nucleotide sequence <SEQ ID 332> is:

1 ATGATGCTTC GTGTCATAGA GCTTCCACTA CTTCTATATA AGCAAGCGTT
 51 GGAGAAGGCT TTTGTACAAT ATAATAGCTA CAAAGCGAAG TTAACCAAGG
 101 TAGAACCTTG CTTTAGAGAG AGCCCTGCCT ATATAACTAG CGAAGAGCGA
 151 CTCCAGAGTT TGGATCAGAC TTAGAACGT GCGTACAAAG AGTACCAGAA
 201 GAGATTCCAG GAGCCTTCAC GTTTGGAATC GGAAGTAAGT GGATGTAGAG
 251 AGCATCTTAG AGAGCAGGTA AAACAATTTG AAACCTCAAGG ACTAGACTTG
 301 ATCAAGAAG AGCTTATTTT TGTTAGTGAT GTGTTATTCC GAAAAATGGT
 351 CAGTTGTCTA GTGTCGACAG TGCATGTICC CTTTATGGAG TTTTATTATG
 401 AGTATTTTGA GTTGCATAGA TTGAGGTTGC GGGCCCAATG GATGGCGAAT
 451 GCCGAGATTT ATAGCAAAGT TAGAAAAGCA TTCCCAGAGA TGTGGAAGGA
 501 GACCTTAGAA AAAGCTAAGG CTCCCAGAGA AGAAGAGTAT TGGTTACTTT
 551 GCGAGGAGAG AAAGAGTAAG GAGAAGCGTT TGATTCTCAA CAAGATAGAG
 601 GCAGCTCAGC AGCGGGTAAA AGATTTAGAA CCTCCTCCTA TTAAAGAGAC
 651 AGGGAAACAG AAACGGAAGA AAGAATATTC GTTTTTCATT CGATTAAAAT
 701 CGTGA

The PSORT algorithm predicts inner membrane (0.1489).

The proteins were expressed in *E.coli* and purified as his-tag and GST-fusion products (Figure 164A; 6444=lanes 11-12; 7391=lanes 2-3; 6413=lanes 4-6). The recombinant protein was used to immunise mice, whose sera were used in Western blots (Figures 164B, 165 & 166) and for FACS analysis.

These experiments show that cp6444, cp6413 & cp7391 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

Example 167 ,
 Example 168 ,
 Example 169 and
 Example 170

The following *C.pneumoniae* protein (PID 4376463) was expressed <SEQ ID 333; cp6463>:

1 MKKKVTIDEA LKEILRLLEGA ATQKEELCAKL LAQGFATTQS SVSRWLRKIQ
 51 AVKVAGERGA RYSLPSSTEK TTTRHLVLSI RHNASLIVIR TVPGSASWIA
 101 ALLDQGLKDE ILGTLAGDDT IFVTPIDEGR LPLLMVSIAN LLQVFLD*

The cp6463 nucleotide sequence <SEQ ID 334> is:

1 ATGAAAAAAA AAGTAACAT AGATGAGGCT TTAAAAGAAA TTTTACGTCT
 51 TGAAGGAGCG GCAACTCAGG AGGAATTATG TGCAAAACTC TTAGCTCAAG
 101 GTTTTGCTAC AACCCAGTCG TCTGTATCTC GTTGGCTACG AAAGATTACG
 151 CCGTAAAGG TTGCTGGAGA GCGTGGTGCT CGTTATTCTT TACCCTCTTC

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201 AACAGAGAAG ACCACGACCC GTCATTTGGT GCTCTCTATT CGCCATAACG
 251 CCTCTCTTAT TGTAATTCGT ACGGTTCCCTG GTTCAGCTTC TTGGATCGCT
 301 GCTTTGTTAG ATCAAGGGCT CAAAGATGAA ATTCCTGGAA CTTTGGCAGG
 351 AGATGACACG ATTTTGTGCA CTCCTATAGA TGAAGGGAGG CTCCCATTGT
 5 401 TGATGGTTTC GATTGCAAAAT TTAGTGCAAG TTTTCTTGGG TTAA

The PSORT algorithm predicts inner membrane (0.1510).

The following *C.pneumoniae* protein (PID 4376540) was also expressed <SEQ ID 335; cp6540>:

1 MSQCQSSSTS TWEWMKSFVP NWKNPTPLS PIPSEDEFIL AYEPFVLPKT
 51 DPENQAQANPP GTSTPNVENG IDDLNPLLQ PNEQNANNP GTSGSNPTSL
 101 PAPERLPETE ENSQEEOQS QNNEDLIG*

The cp6540 nucleotide sequence <SEQ ID 336> is:

1 ATGTCTCAAT GTCAGAGTAG CAGTACATCT ACCTGGGAAT GGATGAAATC
 51 TTTTGTGCCA AACTGGAAGA ATCCAACTCC CCCCTTATCT CCTATACCTT
 101 CTGAGGACGA ATTTATATTA GCATACGAGC CATTTGTTCT ACCGAAAACA
 151 GATCCAGAAA ACGCACCAAGC TAATCCTCCA GGCACATCTA CACCGAATGT
 201 AGAAAACGGG ATCGATGATC TCAACCTCT TCTGGGGCAA CCCAACGAAC
 251 AAAACAATGC CAACAATCCA GGAACCTCTG GATCTAATCC TACATCTCTA
 301 CCCGCCCCCG AACGACTCCC TGAACTGAA GAGAACAGCC AAGAAGAAGA
 351 ACAAGGATCT CAAAATAATG AGGATCTTAT AGGATAA

20 The PSORT algorithm predicts cytoplasm (0.3086).

The following *C.pneumoniae* protein (PID 4376743) was also expressed <SEQ ID 337; cp6743>:

1 LREEGSVSFR EYFRAYMCDK IVAQKNFLFT LDAVIKQAGW RSQEKLNLFY
 51 VESQALGREI KVSLEEYIQS MVGILGSQRT KKSFKFSVDF TPLEQALQER
 101 CSSDDDEDAT ATSTATGATA SPTDMHEDE*

25 The cp6743 nucleotide sequence <SEQ ID 338> is:

1 TTGAGAGAAG AAGGTAGTGT TTCTTTCAGA GAATATTTCA GAGCCTATAT
 51 GTGTGATAAA ATCGTGGCAC AGAAGAACTT CTTATTTACT TTAGACGCTG
 101 TAATTAACA GGCCTGTGG AGATCACAAG AGAACTCAA TTTATTTTAT
 151 GTTGAAAGTC AGGCTTTAGG AAGAGAAATC AAAGTCAGCT TAGAGGAATA
 301 TATTCAGAGT ATGGTCGGGA TTTTGGGATC TCAGAGAACC AAGAAAAGCT
 251 TTAAGTTTTT TGTGCACTTT ACCCTTTAG AGCAGGCTCT ACAAGAAAGA
 301 TGCTCTTCTG ATGATGACGA AGATGCAACA GCAACTTCGA CCGCTACAGG
 351 GGCAACAGCA TCTCCGACTG ACATGCACGA AGATGAGTAA

The PSORT algorithm predicts cytoplasm (0.2769).

35 The following *C.pneumoniae* protein (PID 4377041) was also expressed <SEQ ID 339; cp7041>:

1 MLMLMMIIG ITGGSGAGKT TLTONIKEIF GEDVSVICQD NYYKDRSHYT
 51 PEERANLIWD HPDAFDNDLL ISDIKRLKNN EIVQAPVDF VLGNRSKTEI
 101 ETIYPSKVIL VEGILVFENQ ELRLDMDIRI FVDTDADERI LRRMVRDVQE
 151 QGDSVDCIMS RYLSMVKPMH EKFIETPRKY ADIIIVHGNR QNVVTNLSQ
 40 201 KIKNHLENAL ESDETYMYVN SK*

The cp7041 nucleotide sequence <SEQ ID 340> is:

1 ATGTTGATGA TGCTTATGAT GATTATTGGA ATTACAGGAG GTTCTGGAGC
 51 TGGGAAAACC ACCCTAACCC AAAACATTAA AGAAATTTTC GGTGAGGATG
 101 TGAGTGTTAT CTGCCAAGAT AATTATTACA AAGATAGATC TCATTATACT
 45 151 CCTGAGAAGC GTGCCAATTT AATTGGGAT CATCCGACG CCTTTGATAA
 201 TGACTTATTA ATTTCAAGCA TAAAACGTCT AAAAAATAAT GAGATTGTCC
 251 AAGCCCCAGT TTTTGATTTT GTTTTAGGTA ATCGATCTAA AACGGAGATA
 301 GAAACGATCT ATCCATCTAA AGTTATTCTT GTTGAAGGTA TTCTGGTCTT
 351 TGAAAATCAA GAACTTAGAG ATCTTATGGA TATTAGGATC TTTGTAGACA
 50 401 CCGATGCTGA TGAAAGGATA CTACGCCGTA TGGTTCGAGA TGTTCAGAA
 451 CAAGGAGATA GCGTGGACTG CATCATGTCT CGTTATCTTT CTATGGTAAA
 501 CCCTATGCAT GAGAAATTTA TAGAGCCGAC TCGGAAATAT GCTGATATCA
 551 TTGTACATGG AAATTACCGA CAAAACGTAG TAACAAATAT TTTGTACACG
 601 AAAATTAAAA ATCATTTAGA GAATGCCCTG GAAAGCGATG AGACGTATTA
 55 651 TATGGTCAAC TCTAAGTAA

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The PSORT algorithm predicts inner membrane (0.1022).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 167A; 6463 = lanes 2-4; 6540 = lanes 5-7; 6743 = lanes 8-9; 7041 = lanes 10-11). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 167B, 168, 169 & 170) and for FACS analysis.

These experiments show that cp6463, cp6540, cp6743 & cp7041 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

Example 171 and Example 172 and Example 173

The following *C.pneumoniae* protein (PID 4376632) was expressed <SEQ ID 341; cp6632>:

```

1  VQLFQYMNES GWDWLCDFDS QGEGFQLSRL VGLLHSSWAL YEAKEQFYLP
51  EVSLLTWEEL IEMQLLSKPT KHGVAKDLCN VPEKHFQRFY QYLGSLDLNQ
101 RFENTFLNYP KYHLDR*
```

The cp6632 nucleotide sequence <SEQ ID 342> is:

```

1  GTGCAATTAT TTCAATATAT GAATGAGTCC GGATGGGATT GGCTTTGTGA
51  TTTTGATTCT CAAGGCGAGG GATTCCAGTT ATCACGCTCTG GTTGGGCTGT
101 TACATTCGTC CTGGGCATTA TACGAAGCAA AAGAGCAATT TTACCTTCCT
151 GAGGTTTCTC TATTGACCTG GGAAGAAGTG ATAGAAATGC AGTTATTAAG
201 CAAACCAACA AAACACGGGG TTGCAAAAGA TCTTTGTAAT GTATTGAAA
251 AACACTTTCA AAGGTTTAGA CAGTACCTAG GTTCCTTAGA TCTAAATCAA
301 AGGTTGAAAA ATACCTTCTT GAATTATCCT AAATACCATT TAGATAGGGA
351 GTGA
```

The PSORT algorithm predicts cytoplasm (0.3627).

The following *C.pneumoniae* protein (PID 4376648) was also expressed <SEQ ID 343; cp6648>:

```

1  MPVSSAPLPT SHRPSSGNLG LMEPNKALK AKHQDKTTRT IKLLVKILVA
51  ILVIEVLGII AAFFIPGTPP ICLIIILGGLI LTTVLCVLLL VIKLALVNKT
101 EGTABEQIK RKLSSKSIS*
```

The cp6648 nucleotide sequence <SEQ ID 344> is:

```

1  ATGCCCGTGT CCTCAGCCCC CCTACCCACA AGCCACCGCC CTTCCTCTGG
51  AAATCTAGGC CTCATGGAAC CAAATTCCAA AGCTCTAAAA GCAAAGCATC
101 AAGATAAAAC GACGAAGACG ATTAACCTTT TAGTTAAAAAT CCTTGTGTC
151 ATTCTAGTAA TAGAAGTTT AGGAATAATT GCAGCTTTCT TTATTCCTGG
201 GACTCCTCCC ATCTGCTTGA TTATCCTAGG AGGCCTTATT CTACACACAG
251 TACTCTGTGT GCTTCTTCTT GTTATAAAGC TTGCCCTTGT AAACAAAACC
301 GAAGGAACAA CTGCTGAACA GCAGATAAAA CGTAAACTCT CTCTCTAAAG
351 TATTCTTAG
```

The PSORT algorithm predicts inner membrane (0.6074).

The following *C.pneumoniae* protein (PID 4376497) was also expressed <SEQ ID 345; cp6497>:

```

1  MKPNSIIFLE NTKHYDPDIFR EGFVRDRHGL MEASDWLLST BITIIRSILG
51  AIPILGNILG AGRLYSVWYT SDEDWKQV *
```

The cp6497 nucleotide sequence <SEQ ID 346> is:

```

1  ATGAAGCCAA ATAGTATTAT TTTTITAGAA AATACTAAGC ATTATCCCGA
51  CATCTTTTCGA GAAGGATTTG TTCGTGATCG TCATGGACTA ATGGAAGCCT
101 CGGATTGGTT ACTTTCTACG GAAATTACGA TCATTGCTC CATTCCTGGA
151 GCTATCCCTA TTTTAGGAAA TATTCTTGA GCCGACGAC TCTATAGCGT
```


201 TTGGTATACA AGTGACGAAG ATTGGAAAAA ACAAGTGGTT TGA

The PSORT algorithm predicts inner membrane (0.145).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 171A; 6632 = lanes 5-7; 6648 = lanes 8-10; 6497 = lanes 2-4). The recombinant proteins were used to immunise mice,
5 whose sera were used in Western blots (Figures 171B, 172, 173) and for FACS analysis.

These experiments show that cp6632, cp6648 and cp6497 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

Example 174 ,
10 Example 175 ,
Example 176 ,
Example 177 and
Example 178

The following *C.pneumoniae* protein (PID 4377200) was expressed <SEQ ID 347; cp7200>:

1 MPVPIDNSSR NLQEVPESE DLEQHAEESP THQSARSSSL QLSLASSAIS
51 SRVEQLSSLV LGMENSDFSS LRDVPIPSAI YESSTHTPVP TPLVGVGYN
101 GSQSGYYDTQ RESLHLSQLL GSRRVEVVYN QGNFMEASLL NLCPRRPRRD
151 PSPISLALLE LWBAFFLEHP PGSTFNPIFF W*

The cp7200 nucleotide sequence <SEQ ID 348> is:

1 ATGCCCCGTTT CTATAGATAA TTCCTCTCGC AACCTACAAG AAGTTCAGAG
51 AAGCCTAGAA GACCTCGAAC AACACGCAGA AGAATCTCCT ACTCATCAAA
101 GTGCAGAAAG CAGTCTTTTG CAACTGTCTC TAGCCTCCTC AGCAATTTCT
151 AGTAGAGTAG AACAACTATC TTCCCTCGTC TTAGGAATGG AAAAATTCAGA
201 TTTCTCTCTT TTAAGAGACG TTCCTATCTT CTCAGCTATC TACGAATCTT
251 CAACACACAC ACCTGTCCCC ACTCCTCTAG TTGGCGTGGG ATATATCAAC
301 GGAAGTCAAT CAGGATACTA CGATACACAA AGAGAATCTC TTCACCTCAG
351 CCAATTGTTA GGAAGCCGAA GAGTTGAAGT TGTCTATAAC CAAGGAACT
401 TCATGGAGGC CTCTTTGCTA AATCTGTGCC CCAGAAGACC TCGAAGAGAT
451 CCCTCTCCAA TTTCTTTAGC TCTATTAGAG CTCTGGGAAG CATTTTTTTT
501 AGAACACCCC CCAGGTAGCA CTTTAAATCC AATATTTT TGGTAA

The PSORT algorithm predicts cytoplasm (0.3672).

The following *C.pneumoniae* protein (PID 4377235) was also expressed <SEQ ID 349; cp7235>:

1 LNFVSTLTGS DFYAPVLEKL ERAFADTGTQ VILFSSSPDF IVHPIAQQLG
51 ISSWYASCYR DQSAEQTIYK KCLTGDKKAQ ILSYIKKINQ ARSHTFSDHI
101 LDLPFLMLGE EKTIVVRPQGR LKKMAKKYYW NIV*

The cp7235 nucleotide sequence <SEQ ID 350> is:

1 TTGAATTTTG TATCGACTCT GACCGGCTCC GATTTTATG CTCCTGTTTT
51 AGAAAACTA GAAGAAGCTT TTGCAGATAC CACAGGACAG GTGATCCTTT
101 TTTCTTCTTC TCCAGACTTT ATTGTCCACC CCATAGCGCA GCAACTCGGG
401 ATTAGTTCTT GGTATGCGTC GTGTTATCGC GATCAGTCTG CAGAACAGAC
201 GATCTATAAA AAATGTCTTA CAGGGGATAA AAAAGCGCAA ATTTTGAGTT
251 ATATTAAAAA AATTAATCAA GCAAGAAGCC ATACCTTCTC CGACCATATT
301 TTAGATCTTC CTTTTCTTAT GCTGGGAGAA GAGAAAACCG TCGTTCGCCC
351 TCAGGGACGA CTCAAGAAAA TGGCAAAAAA ATATTACTGG AATATCGTTT
401 AA

The PSORT algorithm predicts cytoplasm (0.3214).

The following *C.pneumoniae* protein (PID 4377268) was also expressed <SEQ ID 351; cp7268>:

1 MMHRYFIPLL ALLIFSPSLV RAELOPSENK KGGWPTQLSC AEGSQLFCKF

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51 EAAYNNAIRE GKPGILVFFS ERPTPEFADL TNGSFSLSSTP IAKGFNVVVL
 101 CPGLISPLDF FHKMDPVILY MGSFLEMPPE VEAVSGPRLC YILIDEQGGA
 151 QCQAVLPLET KN*

The cp7268 nucleotide sequence <SEQ ID 352> is:

5 1 ATGATGCACC GTTATTTTAT TCCTTTATTA GCACTTCTCA TTTTCTCTCC
 51 TTCTTTAGTC AGGGCAGAGC TACAACCAAG TGAAAACAGA AAAGGGGGGT
 101 GGCCTACACA ACTTTCCTGT GCAGAAGGTT CGCAACTCTT CTGTAAATTC
 151 GAAGCTGCCT ATAATAATGC AATTGAGGAA GGGAAACCTG GGATTTTAGT
 201 CTTTTTCTCT GAGCGACCCA CACCAGAATT TGCCGACTTA ACGAATGGTT
 10 251 CATTTTCTCT CTCACGCCA ATCGCCAAGG GCTTTAATGT CGTGTGTTA
 301 TGCCCCGGGC TTATCAGTCC CTTAGACTTT TTCCACAAAA TGGATCCTGT
 351 GATTCTCTAT ATGGGAAGTT TTCTAGAGAT GTTCCCTGAA GTGGAGGCAG
 401 TTAGTGGCCC TCGCTTATGT TATATCTTAA TAGATGAACA GGGTGGGGCT
 451 CAATGTCAGG CTGTCCTGCC TTTAGAAACA AAGAATTAG

15 The PSORT algorithm predicts inner membrane (0.1235).

The following *C.pneumoniae* protein (PID 4377375) was also expressed <SEQ ID 353; cp7375>:

1 MQRILIVGID TGVGKTIVSA ILARALNAEY WKPIQAGNLE NSDSNIVHEL
 51 SGAYCHPEAY RLHKPLSPHK AAQIDNVSIE ESHICAPKTT SNLIETSGG
 101 FLSPCTSKRL QGDVFSWWSW SWILVSQAYL GSINHTCLTV EAMRSRNLNI
 20 151 LGMVVGYPE DEEHWLTQEI KLPIIGTLAK EKEITKTIIS CYAEQWKEVW
 201 TSNHQGIQGV SGTPLSLNLH*

The cp7375 nucleotide sequence <SEQ ID 354> is:

1 ATGCAACGTA TCATCATTTGT AGGAATCGAC ACTGGCGTAG GAAAAACCAT
 51 TGTCAGTGCT ATCCTTGCTA GAGCACTTAA CGCAGAATAC TGGAAACCTA
 25 101 TACAAGCAGG GAATCTAGAA AATTCAGATA GCAATATTGT TCATGAGCTA
 151 TCGGGAGCCT ACTGTCATCC CGAAGCTTAT CGATTGCATA AGCCCTTGTC
 201 TCCACACAAG GCAGCGCAAA TCGATAATGT AAGTATCGAA GAGAGTCATA
 251 TTTGTGCGCC AAAACAACCT TCGAATCTGA TTATTGAGAC TTCAGGAGGA
 301 TTTTATATCC CCTGCACATC AAAAAGACTT CAGGGAGATG TGTTTTCTTC
 30 351 TTGGTCATGT TCTTGGATT TTAGTGAGCCA AGCATATCTC GGAAGTATCA
 401 ATCACACCTG TTTAACGGTA GAAGCAATGC GCTCACGAAA CCTCAATATC
 451 TTAGGTATGG TGGTAAATGG GTATCCAGAG GACGAAGAGC ACTGGCTAAC
 501 TCAAGAAATC AAGCTTCCTA TAATCGGGAC TCTTGCCAAG GAAAAAGAAA
 551 TCACAAAGAC AATCATAAGC TGTTATGCCG AACAAATGAA GGAAGTATGG
 35 601 ACAAGCAATC ATCAGGGAAT TCAGGTGTA TCTGGCACCC CTTCACTCAA
 651 TCTGCATTAG

The PSORT algorithm predicts cytoplasm (0.0049).

The following *C.pneumoniae* protein (PID 4377388) was also expressed <SEQ ID 355; cp7388>:

1 MOVLLSPQLP PPPQHSVSI SSPSKLRVLA ITFLVFGMLL LISGALFRTL
 40 51 GIPGLSAAIS FGLGIGLSAL GGVLMISGLL CLLVKREIPT VRPEEIPGV
 101 SLAPSEEPAL QAAQTLAQL PKELDQLDLD IQEVFACLRK LKDSKYESRS
 151 FLNDAKKELR VFDFVVEDTL SEIFELRQIV AQEGWDLNFI INGGRSLMMT
 201 ABESLDDLPH VSKRLGYLPS GDVRGEGLLK SAKETVARLM SLHCEIHKVA
 251 VAFDRNSYAM AEKAPAKALG ALEESVYRSL TQSYRDKFLE SERAKIPWNG
 45 301 HITWLRDDAK SGCAEKLRD AEERWKKFRK AVFWVREDGG PDINNLLGDW
 351 GTVLDPYRQE RMDEITFHEL YEKTTFLKRL HRKCALAKTT FEKKRSKKNL
 401 QAVEERANARR LKYVRDWDYDQ EFQKAGERLE KLHALYPEVS VSIRENKIQE
 451 TRSNLEKAYE AIEENYRCCV REQEDYWKKE EKREAEFRER GNKILSPEEL
 501 ESSLEQFDHG LKNFSEKLM ELEGHILKLQK EATAEVENKI LSDAESRLEI
 50 551 VFEDVKEMPC RIEIEKTLR MAELPLLPTK KAFKACSQY NSCAEMLEKV
 601 KPYCKBSLAY VTSKERLVSL DEDLRRAYTE CQKRFQDSDG LESEVRACRE
 651 QLRERIQEFB TQGLDLVEKE LLCVSSRLRN TECDCVSGVK KEAPPGKKFY
 701 AQYYDEIYRV RVQSRWMTMS ERLREGVQAC NKMLKAGLSE EDKVLKEEY
 751 WLYREERKNK EKRLVGTKIV ATQQRVAAFE SIEVPEIPEA PEEKPSLLDK
 55 801 ARSLPTREDH T

The cp7388 nucleotide sequence <SEQ ID 356> is:

1 ATGCAAGTAC TTCTATCTCC GCAGCTACCC CCCCCCCCC AACACTCTGT
 51 AGGGTCGATT TCTTCTCCAT CTAAACTTCG CGTTTATAGCG ATTACTTTTT

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101 TAGTTTTTGG TATGCTCTTA CTGATTTTCAG GAGCTCTCTT TCTGACGTTA
 151 GGGATTCCAG GATTGAGTGC AGCAATTTCT TTTGGATTAG GCATCGGTCT
 201 CTCCGCATTA GGAGGAGTGC TGATGATTTC GGGACTACTA TGTC'TTTTAG
 251 TAAAACGAGA GATTCCGACA GTACACCAG AAGAAATTC TGAAGGGGTT
 301 TCGCTGGCTC CTTCTGAGGA GCCAGCTCTA CAGGCAGCTC AGAAGACTTT
 351 AGCTCAGCTG CCTAAGGAAT TGGATCAGTT AGATACAGAT ATTACAGGAAG
 401 TGTTTCGCATG TTTAAGAAAG CTGAAAGATT CTAAGTATGA AAGTCGAAGT
 451 TTTTAAACG ATGCTAAGAA GGAGCTTCGA GTTTTTCGACT TTGTGGTTGA
 501 GGATACCCCTC TCGGAGATT TCGAGTTGCG GCAGATTGTG GCTCAAGAGG
 551 GATGGGATT TAACT'TTTTG ATCAATGGGG GACGAAGCCT CATGATGACT
 601 GCAGAACTCG AATCGCTTGA TTTGTTTCAT GTATCGAAGC GGCTAGGGTA
 651 TTTACCTTCT GGGGATGTT CAGGGGAGGG GTTAAAGAA TCTGCCAAGG
 701 AGATAGTCGC TCGTTTGATG AGCTTGCATT GCGAGATTCA CAAGGTGGCG
 751 GTATCGGTTG ATAGGAATTC CTATGCGATG GCAGAAAAGG CGTTTTCGAA
 801 AGCGTTGGGA GCTTTAGAAG AGAGTGTGTA TCGGAGTCTG ACGCAGAGTT
 851 ATAGAGATAA ATTTTGGAG AGCGAGAGGG CGAAGATCCC ATGGAATGGG
 901 CATATAACCT GGTAAAGAGA TGATGCGAAG AGTGGGTGTG CTGAAAAGAA
 951 GCTTCGGGAT GCCGAGGAAC GTTGAAGAA ATTTAGGAAA GCAGTCTTTT
 1001 GGGTAGAAGA AGACGGGGGC TTTGACATCA ATAATCTCCT TGGAGACTGG
 1051 GGGACAGTGC TTGATCCTTA TAGACAAGAG AGAATGGACG AGATAACGTT
 1101 CCATGAGTTG TATGAAAAA CTACGTTTTC GAAAAGACTG CACAGAAAGT
 1151 GTGCGTTAGC GAAAACAACC TTTGAAAAGA AGAGATCTAA AAAGAATTTG
 1201 CAGGCAGTCG AGGAGGCGAA TGCAGCTAGG TTGAAATATG TAAGGGATTG
 1251 GTATGATCAG GAGTTTCAGA AAGCAGGGGA GAGATTAGAG AAATGTCATG
 1301 CTTTGTATCC TGAGGTTTCA GTCTCTATAA GAGAGAACAA AATACAAGAG
 1351 ACGCGCTCTA ATTTAGAGAA AGCCTATGAG GCTATCGAAG AGAACTATCG
 1401 TTGCTGTGTC CGAGAGCAAG AGGACTACTG GAAAGAAGAA GAGAAAAGGG
 1451 AAGCGGAGTT TAGGGAGAGG GGAACAAGA TTC'TTCTCC TGAGGAGCTG
 1501 GAAAGTTCTT TGGAGCAATT CGACCATGGT TTGAAAAATT TTTCTGAGAA
 1551 ATTAATGGAA TTGGAAGGGC ATATCTTAAA ACTTCAGAAA GAAGCCACAG
 1601 CAGAGGTGGA GAATAAAATA CTTTCAGATG CAGAGAGCCG CTTGAGATT
 1651 GTATTTGAAG ATGTCAAGGA GATGCCCTGT CGAATTGAGG AGATAGAGAA
 1701 GACGCTGCGT ATGGCGGAGC TGCCCTACT TCCTACGAAG AAGGCGTTTG
 1751 AGAAGGCCCTG CTCACAATAT AATAGCTCGC CAGAGATGTT GGAGAAGGTG
 1801 AAGCCTTACT GCAAGGAGAG CCTCGCCTAT GTGACTAGCA AAGAGCGTTT
 1851 AGTGAGCTTG GATGAAGATT TACGACGAGC CTACACAGAG TGTCAGAAGA
 1901 GATTCCAGGG GGATTCCGGT TTGGAGTCGG AAGTAAGAGC CTGTGAGAG
 1951 CAATGCGGAG AGCGGATCCA AGAGTTTGAA ACTCAAGGGC TGGACTTGGT
 2001 GGAAAAAGAG TTGCTTTGTG TGAGTAGTAG ATTAAGAAAT ACAGAGTGCG
 2051 ATTGTGTATC TGGTGTTAAG AAAGAAGCAC CTCCTGGTAA GAAGTTTAT
 2101 GCCCAGTATT ATGATGAGAT TTATCGAGTT AGAGTTCAAT CCCGATGGAT
 2151 GACGATGTCT GAGAGATTGA GAGAGGGAGT TCAAGCATGC AACAGATGT
 2201 TGAAGGCAGG CCTAAGCGAA GAAGATAAGG TTC'TTAAAGA AGAAGAGTAT
 2251 TGGTTGTATC GAGAGGAGAG AAAGAATAAA GAGAAACGTT TGGTTGGTAC
 2301 TAAGATAGTA GCAACGCAGC AGCGAGTTGC AGCATTTGAA TCCATAGAAG
 2351 TTCTGAGAT TCCTGAGGCC CCAGAGGAGA AACCGAGTTT GCTGGATAAA
 2401 GCGCGTCTT TATTACTCG CGAGGACCAT ACCTAG

The PSORT algorithm predicts inner membrane (0.461).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 174: 7200=lanes 2-3; 7236=lanes 4-5; 7268=lanes 6-8; 7375=lanes 9-10; 7388=lanes 11-12). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 174, 175, 176, 177 & 178) and for FACS analysis.

These experiments show that cp7200, cp7235, cp7268, cp7375 & cp7388 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

Example 179

The following *C.pneumoniae* protein (PID 4376723) was expressed <SEQ ID 357; cp6723>:

-181-

1 MATSVAPSPV PESSPLSHAT EVLNLPNAYI TQPHPIPAAP WETFRSKLST
 51 KHTLCFALTL LLTLGGTISA GYAGYTGNIW ICGIGLGIIIV LTLILALLLA
 101 IPLKNKQTGT KLIDBISQDI SSIGSGFVQR YGLMFSTIKS VHLPELTTQN
 151 QEKTRILNEI EAKKBSIQNL ELKITECQNK LAQKQPKRKS SQKSFMRSIK
 5 201 HLSKNPVILF DC*

The cp6723 nucleotide sequence <SEQ ID 358> is:

1 ATGGCAACTT CCGTAGCCCC ATCACCAGTC CCCGAGAGCA GCCCTCTCTC
 51 TCATGCTACA GAAGTTCTCA ATCTTCCTAA TGCTTATATT ACGCAGCCTC
 101 ATCCGATTCC AGCGGCTCCT TGGGAGACCT TTCGCTCCAA ACTTTCCACA
 151 AAGCATACGC TCTGTTTTGC CTTAACTACTA CTGTTAACCT TAGGGGGAAC
 201 GATCTCAGCA GGTACCGCAG GATATACTGG AAACCTGGATC ATCTGTGGCA
 251 TCGGCTTGGG AATTATCGTA CTCACACTGA TTCTTGCTCT TCTTCTAGCA
 301 ATCCCTCTTA AAAATAAGCA GACAGGAACA AAACCTGATTG ATGAGATATC
 351 TCAAGACATT TCCTCTATAG GATCAGGATT TGTTTCAGAGA TACGGGTTGA
 15 401 TGTTCTCTAC AATTAAAAGC GTGCATCTTC CAGAGCTGAC AACACAAAAT
 451 CAAGAAAAAA CAAGAATTTT AAATGAAATT GAAGCGAAAA AGGAATCGAT
 501 CCAAAATCTT GAGCTTAAAA TTAGTGAAGT CCAAAACAAG TTAGCACAGA
 551 AACAGCCGAA ACGGAAATCA TCTCAGAAAT CATTATGCG TAGTATTAAG
 601 CACCTCTCCA AGAACCTGT AATTTTGTTC GATTGCTGA

20 The PSORT algorithm predicts inner membrane (0.6095).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 179A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 179B) and for FACS analysis.

25 These experiments show that cp6723 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 180

The following *C.pneumoniae* protein (PID 4376749) was expressed <SEQ ID 359; cp6749>:

1 MSYYPSLWYL KVQHPQAAF DFTRSLCSRI SNFALGVIAL LPIIGQLYVG
 51 LDWLLSRIKK PEFPDSDVDQI VRVEHVVGHD HRSRVEDILK RQRLSLEPRD
 30 101 EGKVHGDLPs APFF*

The cp6749 nucleotide sequence <SEQ ID 360> is:

1 ATGAGTTATT ACTTTTCTCT TTGGTATCTG AAGGTGCAAC AGCACTTTCA
 51 AGCAGCATTT GATTTTACTC GCTCCCTGTG TTCACGAATT TCTAATTTTG
 101 CTTTGGGAGT GATTGCATTG CTTCTTATTA TTGGGCAGTT GTATGTAGGG
 35 151 CTGGACTGGC TCCTCTCTAG GATAAAAAAG CCAGAATTTT CTTCCGATGT
 201 GGATCAGATC GTGCGAGTAG AACACGTCGT GGGTCACGAC CATAGAAGTC
 251 GAGTTGAAGA TATTCTAAAG AGACAAAGGC TCTCATTAGA GCCTAGAGAC
 301 GAGGGGAAGG TTCACGGAGA TCTGCCTTCA GCTCCTTTTT TTGA

The PSORT algorithm predicts inner membrane (0.2996).

40 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 180A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 180B) and for FACS analysis.

These experiments show that cp6749 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 181 ,
 Example 182 ,
 Example 183 ,
 Example 184 and
 Example 185

The following *C.pneumoniae* protein (PID 4376301) was expressed <SEQ ID 361; cp6301>:

```

1  LNQDLQNVYQ ECQKATGLES EVSAYRDHLR EQITEFETQG LDVIKELLF
51  VSSTLKSKLS YDPLIADIPC MKFYEEFYDG IDKARVQSRW LEKSERYRKA
101 KKGFPQEMLKE GLFKEDQALK KAEYRLLREK RMNKEKLLIC NKIEAAQQRV
151 QEFGPSDS*

```

The cp6301 nucleotide sequence <SEQ ID 362> is:

```

1  TTGAATCAGG ATTTACAAAA TGTATACCAA GAGTGCCAGA AGGCTACAGG
51  TTTAGAATCG GAAGTGAGTG CATATAGAGA TCATCTTAGA GAGCAGATCA
101 CAGAGTTTGA AACTCAAGGG CTGGACGTGA TAAAAGAAGA ACTTCTTTT
151 GTGAGTAGTA CTCTCAAAAG TAAATTGAGC TATGATCCAT TAATAGCAGA
201 CATTCCCTGT ATGAAGTTT ATGAGGAGTA TTATGATGGC ATTGATAAAG
251 CGAGAGTTCA ATCCCAGATG CTGGAGAAGT CTGAGAGGTA TAGAAGGCG
301 AAGAAGGGAT TCCAAGAGAT GCTGAAGGAA GGCCTATTCA AAGAAGATCA
351 GGCTTTGAAA AAAGCAGAGT ATAGATTACT TCGAGAGAAG AGAATGAATA
401 AGGAGAAGCT TTTGATTTC AATAAGATAG AAGCAGCTCA GCAGCGAGTC
451 CAAGAATTG GACCTCGGA TTCATAA

```

The PSORT algorithm predicts cytoplasm (0.4621).

The following *C.pneumoniae* protein (PID 4376558) was also expressed <SEQ ID 363; cp6558>:

```

1  MNIPAPQVPV IDEPVVNNTS SYGLSLKSSL RPITYLILAI LAIATLMSVL
51  YFCGIISVGT FVLGMLIPLS VCSVLCVAYL FYQSSIEKT KVFSITSPSV
101 FFSDEDLNLL LGREEDSVSA IDELLKNFPA DDFRRPKMLP YSNFLDEQGR
151 PNESREEDSH TSKIL*

```

The cp6558 nucleotide sequence <SEQ ID 364> is:

```

1  ATGAACATAC CCGCTCCCCA AGTACCAGTC ATAGATGAGC CTGTAGTGAA
51  CAACACAAGT AGCTATGGTC TTTCATTGAA AAGTAGTTTA AGACCGATTA
101 CTTATTGAT TTTAGCTATC TTAGCTATAG CCACACTGAT GTCTGTCTC
151 TACTTTTGTG GCATCATTAG TGTGGGACG TTTGTTTGG GCATGCTGAT
201 CCCTCTATCG GTCTGCTCTG TTCTTTGCGT TGCCTATTTA TTCTATCAGC
251 AATCTTCTAT AGAAAAGACT AAGGTCTTTT CTATAACCAG TCCTTCAGTA
301 TTTTCTCTG ATGAGGATCT TAATTACTC TTAGGTCGAG AAGAAGATTC
351 AGTGCTGCA ATTGATGAAC TTCTTAAGAA CTTTCCAGCT GATGATTTC
401 GTAGCCGAA GATGCTTCCT TATTCAAATT TTCTAGATGA GCAGGGAAGG
451 CCTAATGAGA GTAGGAAGA AGACTCTCAT ACTTCCAAGA TCTTATAA

```

The PSORT algorithm predicts inner membrane (0.4630).

The following *C.pneumoniae* protein (PID 4376630) was also expressed <SEQ ID 365; cp6630>:

```

1  MSMTIVPHAL FKNHCECHST FPLSSRTIVR IAIASLFCIG ALAALGCLAP
51  PVSIVVGSVL AFIAFVILSL VILALIFGEK KLPPTPRIIP DRFTHVIDEA
101 YGLSISAFVR EQQVTLAEFR QFSTALLCNI SPEEKIKQLP SELRSKVESF
151 GISRLAGDLE KNNWPIFEDL LSQTCPLYWL QKFISAGDPQ VCRDLGVPRE
201 CYGYWLGPL GYSTARATIF CKETHHILQQ LTKEDVLLK NKALQEKWDT
251 DEVKAIVERI YTTYTARGTL KTEAGGLTKE TISKELLLLS LHGYSFDQLQ
301 LITQLPRDAW DWLCFVDNST AYNLQLCALV GALSSQNLLD ESSIDFDVNL
351 GLYVIQDLKE AVQAFSASDE PKKELGKFL RHLSSVSKRL ESVLROGLHR
401 IALEHGNARA RYVDVNFVTG ARIHRKTSIF FKD*

```

The cp6630 nucleotide sequence <SEQ ID 366> is:

```

1  ATGAGCATGA CGATCGTTCC ACATGCTTTA TTTAAAAATC ATTGCGAGTG
51  TCATTCTACC TTTCTTTTGA GTTCAAGGAC TATTGTAAGA ATAGCCATTG
101 CCAGCCTCTT TTGTATAGGT GCATTAGCAG CTTTAGGCTG TTTGGCTCCT
151 CCCGTTCTT ATATTGTTGG GAGTGTTTGA GCTTTATTG CTTTGTTCAT
201 TCTTTCTTTA GTAATTTTAG CTTTGATTTT TGGAGAGAAG AAGCTTCCAC

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251 CAACACCAAG AATCATTCCT GATAGATTTA CTCACGTGAT AGATGAAGCT
 301 TATGGCCTTT CAATCTCTGC ATTTGTAAGA GAACAGCAGG TAACATTAGC
 351 CGAGTTTAGA CAATTTTCTA CTGCCCTGTT GTGTAACATA TCTCCTGAAG
 401 AGAAATCAA ACAATTGCCT TCTGAATTGC GAAGTAAAGT AGAGAGTTTT
 451 GGTATTAGCA GGCTCGCAGG TGATTAGAA AAGAATAATT GGCCAATATT
 501 TGAAGATCTT TTAAGCCAAA CCTGCCCGTT ATATTGGCTT CAGAAATTTA
 551 TATCAGCAGG AGATCCACAA GTTTGTAGAG ACCTAGGTGT CCCTAGAGAA
 601 TGTATGGGT ACTATTGGCT AGGGCCTTTG GGATACAGTA CAGCTAAGGC
 651 TACAATTTTT TGTAAGAGA CGCATCATAT TCTTCAACAA TTAACGAAAG
 701 AGGAGCTTCT TTTATTAAAA AACAAGGCTC TTCAAGAGAA ATGGGATACT
 751 GATGAAGTCA AAGCAATTGT AGAGCGTATC TACACTACCT ATACGGCAGC
 801 AGGAACTCTA AAGACCGAAG CAGGGGGACT TACAAAGAG ACAATCAGTA
 851 AGGAATTGCT ATTGTGAGC TTGCATGGCT ATTCITTTGA TCAGCTACAG
 901 CTGATCACTC AACTTCCTAG AGATGCTTGG GATTGGCTGT GTTTTGTAGA
 951 TAACAGTACC GCATACAACC TTCAGCTTTG TGCTCTTGTG GGAGCTTTGT
 1001 CATCCCAAAA TCTTCTTGAC GAATCTTCTA TCGATTTTGA TGTAAACCTA
 1051 GGCCTGTATG TGATTTCAGGA TCTAAAAGAA GCTGTTCAGG CATTITCTGC
 1101 TTGATGATGAG CCAAAGAAAG AACTAGGTAA ATTCITGTGA AGGCATTTGA
 1151 GTTCAGTTTC TAAGCGATTA GAGAGTGTAT TAAGACAGGG TCTTCACAGA
 1201 ATAGCTCTAG AGCATGGAAG TGCCAGAGCT AGGGTTTATG ACGTCAATTT
 1251 TGTAACAGGA GCTAGAATTC ATAGGAAGAC GAGTATCTTC TTTAAAGACT
 1301 AA

The PSORT algorithm predicts inner membrane (0.7092).

The following *C.pneumoniae* protein (PID 4376633) was also expressed <SEQ ID 367; cp6633>:

25 1 MVNIQPVYRN TQVNSQATQ FSVCQPALSL IIVSVVA AVL AIVALVCSQS
 51 LLSIELGTAL VLVSLILPAS AMFMIYKMRQ EPKELLIPKK IMELIQEHYP
 101 SIVVDIFRDQ EVSIYIEIHL ISILNKTNVF DKAPVYLQEK LLQFGIERFK
 151 DVHPGSKLPNF EBILLQHCPL HWLGRLVYPM VSDVTPGTYG YWCGPLGLY
 201 ENAPSLFERR SLLLLKKISF GEFALLEDGL KKNTWSSSEL VQIRQNLFTR
 251 YYADKEEVDE ABLNADYEQF DSSLHLIFSH KLS*

The cp6633 nucleotide sequence <SEQ ID 368> is:

1 ATGGTTAATA TACAGCTGT GTATAGGAAT ACCCAAGTCA ACTATAGTCA
 51 GGCTACCCAA TTTTCGGTGT GCCAGCCAGC GCTTAGCCTG ATTATCGTTT
 101 CTGTTGTTGC TGCTGTACTC GCTATTGTAG CTTTGGTATG CAGTCAATCT
 151 CTTTATATCCA TAGAGTTAGG AACTGCTCTT GTTCTAGTTT CTCTTATCT
 201 TTTTGTCTCT GCTATGTTTA TGATTATATA GATGAGACAA GAACCTAAGG
 251 AGTTGCTGAT CCCTAAGAAA ATCATGGAAC TCATCCAAGA ACATTATCCA
 301 AGTATTGTTG TTGATTTTAT TAGAGATCAG GAGGTTTCCA TTTATGAGAT
 351 ACATCACTTG ATCTCTATTC TTAATAAGAC GAATGTTTTC GACAAAGCAC
 401 CAGTATATTT ACAAGAAAAA CTCTTACAGT TTGGCATTGA GAAGTTCAAA
 451 GATGTACATC CAAGTAAGCT CCCTAATTTT GAAGAAATTC TTCTACAGCA
 501 TTGCCCATTG CATTTGGTTGG GACGCTCTGGT ATATCCCATG GTATCGGATG
 551 TCACTCCAGG AACCTATGGA TACTATTGGT GTGGTCTTTT AGGACTGTAC
 601 GAGAACGCTC CCTCTCTTTT TGAACGTCGA TCTCTCTAT TGTAAAGAA
 651 AATTAGCTTT GGAGAGTTTG CTCTTTTAGA AGATGGTCTC AAGAAAAACA
 701 CGTGGAGTTC TTCGGAATTC GTTCAAATCA GACAAAACCT TTTTACAAGA
 751 TATTATGCTG ATAAAGAAGA GGTAGATGAA GCAGAGTTAA ACGCTGATTA
 801 CGAACAGTTT GATTCCCTCC TTCACCTTAT TTTTCTCTAC AAGCTCTCTT
 851 GA

50 The PSORT algorithm predicts inner membrane (0.7283).

The following *C.pneumoniae* protein (PID 4376642) was also expressed <SEQ ID 369; cp6642>:

1 MATISPISLT VDHPLVDTKK KSCSNFDKIQ SRILLITAIF AVLVTIGTLL
 51 IGLLLNIPVI YFLTGISFIA VVLSNFILYK RATTLLKPRA CGKHKEIKPK
 101 RVSTNLQYSS ISIAINRSKE NWEHQPKDLQ NLPAPSALLT DNPYEIWKAK
 151 ISLFLSVSLP PGNPEHLI SASENLGKTL LIEETSQNAF ISSYVDTPS
 201 PKSLINEAIQ ETRVEINTEL PAGDSGERLY WQPDFRGRVF LPQIPTTPEA
 251 IYQYYVALYV TYIQTAINTN TQIIQIPLYS LREHLYSREL PPQSRMQQSL
 301 AMITAVKYMA ELHPEYPLTI ACVERSLAQL PQESIEDLS*

The cp6642 nucleotide sequence <SEQ ID 370> is:

60 1 ATGGCTACAA TCTCACCCAT ATCTTTAACT GTAGATCATC CCCTAGTAGA

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51  CACTAAAAAA AAATCCTGCA GCAACTTTGA TAAGATTCAG TCTCGAATTC
101  TATTGATTAC TGCAATCTTT GCTGTCTTAG TTAATATAGG GACCCCTACTT
151  ATTGGGTTGC TTTTAAATAT TCCTGTATATC TATTTCTCTCA CAGGAATTTTC
201  ATTTATTGCT GTTGTCTTAA GCAACTTTAT CCTTTATAAA CGAGCAACCA
5  251  CCCTCTTAAA ACCGCGTGCT TGTGGCAAAC ACAAAGAAAT AAAACCAAAA
301  AGGGTCTCCA CCAACCTACA GTATTCTTCT ATCTCTATCG CAATCAATCG
351  TTCTAAAGAA AACTGGGAAC ACCAACCCTA GGACCTACAG AATCTCCCCG
401  CACCCTCTGC ATTACTCACA GATAACCCCTT ACGAGATATG GAAAGCTAAA
451  CATTCCTGT TTTCCCTAGT ATCCCTCCTA CCGGGAGGCA ATCCAGAACA
10 501  TCTCTTAATT TCAGCTTCCG AAAATTTAGG AAAGACTCTG TTAATTGAAG
551  AAACCTCGCA AAATGCGCCT ATATCTCCTT ACGTAGATAC CACTCCCTCC
601  CCAAAATCCT TGCTCAATGA GGCAATTCAG GAAACCAGGG TAGAAATAAA
651  TACAGAACTC CCTGCGGGAG ATTCAAGAGA ACGTTTATAC TGGCAACCCG
701  ATTTCCGAGG CCGCGTCTTC CTCCCACAAA TACCAACAAC TCCTGAAGCC
15 751  ATCTACCAAT ACTACTATGC ACTCTATGTC ACTTATATCC AGACTGCGAT
801  CAATACGAAC ACCCAAATTA TCCAAATCCC TTTATACAGC TTGAGGGAGC
851  ATCTCTATTC TAGAGAATTG CCCCAGCAAT CAAGAATGCA ACAATCTTTG
901  GCTATGATTA CAGCAGTAAA ATACATGGCC GAGCTGCACC CAGAATATCC
951  GCTAACTATT GCTTGTGTTG AAAGATCCTT AGCCCACTA CCTCAAGAAA
20 1001 GTATTGAGGA TCTCTCTTAG

```

The PSORT algorithm predicts inner membrane (0.5288).

The proteins were expressed in *E.coli* and purified as GST-fusion products. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 181-185) and for FACS analysis.

25 These experiments show that cp6301, cp6558, cp6630, cp6633 and cp6642 are surface-exposed and immunoaccessible proteins, and that they are useful immunogens. These properties are not evident from their sequences alone.

Example 186

The following *C.pneumoniae* protein (PID 4376389) was expressed <SEQ ID 371; cp6389>:

```

30 1  MSEVKPLFLK NDSFDLATQR FQNLINMLQE QAEIYNEYEE KNARVQNEIK
51  EQKDFVKRCI EDFEARGLGV LKEELASLIR DFHDKAKAET SMLIEPCIG
101 FYYSIHQEEQ RQRQERLQKM AERYRDCKQV LEAVQVEQKD MISSRVVVDD
151 SYFEEKEEQ KVDNRKKEQD *

```

The cp6389 nucleotide sequence <SEQ ID 372> is:

```

35 1  ATGTCAGAAG TGAAGCCTTT GTTTTAAAG AATGACTCTT TTGATTTGGC
51  AACTCAGAGA TTCCAGAATC TAATTAACAT GCTACAAGAG CAAGCCGAGA
101 TATATAACGA GTATGAAGAA AAGAATGCTA GGGTTCAGAA TGAGATTAAG
151 GAGCAAAAGG ACTTTGTGAA AAGATGCATA GAGGACTTTG AAGCCAGAGG
201 ACTGGGGGTG CTAAGAAGAG AGCTTGCATC TTTGACGCGT GATTTCATG
40 251  ATAAAGCAAA AGCAGAGACT TCTATGCTCA TTGAATGTCC TTGTATPGGT
301  TTTTATTATA GTATTTCATCA GGAGGAACAA AGGCAAAGGC AAGAAAGGCT
351  TCAAAAGATG GCTGAGCGCT ATAGGGACTG TAAACAAGTC TTGGAGGCTG
401  TCCAGGTGGA GCAAAAAGAT ATGATATCTT CTAGAGTCGT TGTCGATGAC
451  AGCTACTTTG AAGAAGAAAA AGAAGAACAA AAGGTGGATA ACAGAAAGAA
45 501  AGAACAGGAC TAG

```

The PSORT algorithm predicts cytoplasm (0.3193).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 186A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 186B) and for FACS analysis.

These experiments show that cp6389 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 187

The following *C.pneumoniae* protein (PID 4376792) was expressed <SEQ ID 373; cp6792>:

```

5      1  VLQEHFFLSE DVITLAQQLL GHKLITTHEG LITSGYIVET EAYRGPDDKA
      51  CHAYNYRKTQ RNRAMYLKGG SAYLYRCYGM HLLNVTGTP EDIPHAVLIR
     101  AILPDQGKEL MIQRQWRDK PPHLLTNGPG KVCQALGISL ENNRQRLNTP
     151  ALYISKEKIS GTLTATARIG IDYAQEYRDV PWRFLSPED SGKVLS*

```

The cp6792 nucleotide sequence <SEQ ID 374> is:

```

10      1  GTGTACAAG AACATTTTTT TCTATCGGAA GATGTAATTA CACTAGCGCA
      51  ACAGCTTTTA GGACATAAAC TCATCACAAC ACATGAGGGT CTGATAACTT
     101  CAGGTTACAT TGTAGAAACC GAAGCGTATC GTGGCCCTGA TGACAAAGCA
     151  TGCCACGCCT ACAACTACAG AAAAATCAG AGGAACAGAG CGATGTACCT
     201  GAAAGGAGGC TCTGCTTACC TCTACCGTTG CTATGGCATG CATCACCTAT
15      251  TGAATGTTGT CACTGGACCT GAGGACATTC CCCATGCCGT CCTGATCCGG
     301  GCCATCCTTC CTGATCAAGG CAAAGAACTT ATGATCCAAC GCCGCCAATG
     351  GAGAGATAAA CCCCACACC TTCTACCAA TGGACCCGGA AAAGTGTGCC
     401  AAGCTCTAGG AATCTCTTTG GAAAACAATA GGCAACGCCT AAATACCCCA
     451  GCTCTCTATA TCAGCAAAGA AAAAATCTCT GGGACTCTAA CAGCAACTGC
20      501  CCGGATCGGC ATCGATTATG CTCAAGAGTA TCGTGATGTC CCATGGAGAT
     551  TTCTCTATC CCCAGAAGAT TCGGAAAAG TTTTATCTTA A

```

The PSORT algorithm predicts cytoplasm (0.180).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 187A; lanes 2-4).

The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 187B) and for FACS analysis.

These experiments show that cp6792 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 188

The following *C.pneumoniae* protein (PID 4376868) was expressed <SEQ ID 375; cp6868>:

```

30      1  MVETVLHNFQ RYLSKYLYRV FRFPCRKKTF LSSHRVLARP SFPVDYCPGK
      51  IYDLQEIYEE LNAQLFQGA LRLQIGWFRK ATRRGKSVVL GLFHENEQLI
     101  RIHRSLDRQE IPRPFMEYLV YHEMVHSVVP REYSLSGRSI FHGKKFKEYE
     151  QRFPDYDRAV AWEKANAYLL RGYKKRVGGG YGRA*

```

The cp6868 nucleotide sequence <SEQ ID 376> is:

```

35      1  ATGGTTGAAA CAGTACTTCA TAATTTCCAA CGTTATCTGA GCAAGTATCT
      51  CTATAGGGTA TTTTCGCTTC CATGTCGTAA AAAGACGTTT CTATCTTCGC
     101  ACAGGGTTCT TGCTCGTCCT TCATTTCCAG TAGACTACTG TCCGGGAAAG
     151  ATCTATGATT TGCAGGAGAT CTATGAGGAA TTGAATGCGC AGTTATTTC
     201  AGGTGCACTG CGTTTACAGA TTGGTTGGTT CGGAAGGAAA GCTACCAGAA
40      251  AAGGCAAGAG TGTTCCTTTG GGATTGTTTC ATGAAAATGA ACAGTTAATT
     301  CGAATTCATC GTTCTTTAGA TCGGCAGGAA ATCCCAAGAT TTTTATGGA
     351  ATATCTTGTG TATCATGAAA TGGTTCATAG TGTAATCCCT AGAGAGTATT
     401  CTCTATCGGG GCGTTCGATT TTTCATGGTA AAAAGTTTAA AGAATACGAA
     451  CAACGTTTCC CTTGTATGA TCGTCTGTTT GCTTGGGAAA AGGCAAACGC
45      501  TTATTATTATG CGAGGTATA AAAAAAGAGT AGGTGGAGGA TATGGCAGGG
     551  CATAG

```

The PSORT algorithm predicts bacterial cytoplasm (0.325).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 188A; lanes 2-3). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 188B) and for FACS analysis.

These experiments show that cp6868 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 189

The following *C.pneumoniae* protein (PID 4376894) was expressed <SEQ ID 377; cp6894>:

```

1 MYKRCVLDKI LKGIVAGSLI LLYWSSDLLE RDIKSIKGNV RDIQEDIREI
51 SRVVKQQQTS QAIIPAAPGVM LAPKLVRDEA FALLFGDPSY PNLLSLDPYK
101 QQTLPPELLGT NFHPHGILRT AHVGKPENLS PFNGFDYVVG FYDLCIPSLA
151 SPHVKGKYEEL SPDLAVKIEE HLVEDGSGDK EFHIYLRPNV FWRPIDPKAL
201 PKHVQLDEVF QRPHPVTAHD IKFFYDAVMN PYVATMRAVA LRSCYEDVVS
251 VSVENDLKLK VRWKAHTVIN REGKEERKVL YSAFNTLSL QPLPRFVYQY
301 FANGEKIIED ENIDTYRTNS IWAQNFTMHW ANNYIVSCGA YYFAGMDDEK
15 351 IVFSRNPDFY DPLAALIDKR FVYFKESTDS LFQDFKTGKI DISYLPNQR
401 DNFYSFMKSS AYNKQVAKGG AVRETVSADR AYTYIGWNCF SLFFQSRQVR
451 CAMNMAIDRE RIIBQCLDQG GYTISGPFAS SSPSYNKQIE GWHYSPREAA
501 RLLEEGEWID TDGDGIREKV IDGVIVPFRF RLCYVKSVT AHTIADYVAT
551 ACKBIGIECS LLGLDMADLS QAFDEKNFDA LLMGWCLGIP PEDPRALWHS
20 601 EGAMERKGSAN VVGFFHNEAD KIIDRLSYEY DLKERNRLYH RFHEITHREA
651 PYAFLEFSRHC SLLYKDYVKN IFVPHTRTDL IPEAQDETVN VTMVWLEKKE
701 DPCLSTS*

```

The cp6894 nucleotide sequence <SEQ ID 378> is:

```

1 ATGTATAAAA GATGTGTGCT AGATAAAATT TTAAAGGGGA TTGTCCGCCG
25 51 TTCTTTAATT TTGTTTACTT GGTCTCAGCA CCTACTTGAA AGAGACATTA
101 AGTCGATAAA AGGTAACGTA AGAGATATTC AAGAAGACAT TCGTGAAATC
151 TCACCGCTAG TGAAACAACA GCAGACATCA CAAGCTATCC CTGCGGCACC
201 TGGGGTAGTG CTCGCTCCTA AGCTCGTCAG AGACGAAGCT TTTGCTCTAC
30 251 TCTTTGGAGA TCCTAGTTAT CCTAATTTAC TTTCCCTAGA CCCCTATAAA
301 CAGCAGACTC TTCCTGAAC TCTAGGAACA AATTTCCACC CTCATGGTAT
351 CCTACGCACT GCCCATGTCG GAAACCCCGA AAATCTGAGC CCTTTTAATG
401 GCTTTGATTA TGTCTGGGCG TTTTACGATC TCTGTATTCC TAGTTTAGCT
451 TCTCCCCACG TAGGGAATAA CGAAGAATTT TCTCCAGATC TCGCTGTGAA
501 AATAGAAGAA CATCTTGTGG AAGATGGTTC TGGGGATAAA GAGTTTCACA
35 551 TCTATCTGAG GCCGAATGTT TTTTGGCGTC CTATAGATCC TAAGGCCCTT
601 CCAAAACACG TTCAGTTAGA CGAAGTATTT CAACGTCCTC ATCCTGTGAC
651 AGCTCATGAT ATTAAGTTT TCTACGACGC TGTATGAAC CTTATGTAG
701 CAACCATGCG AGCAGTGGCT CTGCGCTCTT GTTATGAAGA TGTGGTTTCT
40 751 GTCTCAGTAG AAAACGATTT AAAATTAGTA GTCAGATGGA AAGCACACAC
801 GGTAATCAAT GAAGAAGGAA AGGAAGAGCG CAAAGTGCTC TACTCTGCAT
851 TTTCTAATAC CTTAAGCTTG CAGCCCCTCC CTAGATTTGT ATATCAGTAT
901 TTTGCTAACG GGGAAAAAAT CATTGAAGAT GAGAATATCG ATACCTACCG
951 AACCAATTCC ATTTGGGCGC AAAACTTCAC TATGCATTGG GCAAACAAC
1001 ATATTTGTAAG TTGTGTGAGC TACTACTTTG CAGGGATGGA TGATGAGAAA
45 1051 ATCGTGTGTT CTAGAAATCC TGACTTCTAT GATCCTCTTG CGGCTCTTAT
1101 TGACAAGCGT TTCGTCTATT TTAAGGAAAG CACAGACTCC CTATTCCAAG
1151 ATTTTAAGAC AGGGAAAATA GACATCTCTT ACCTTCCACC CAACCAAAGA
1201 GATAATTTCT ATAGTTTAT GAAAAGCTCC GCTTATAACA AACAGGTAGC
1251 TAAGGGAGGA GCCGTCCTGT AAACAGTCTC AGCAGATCGA GCATATACGT
50 1301 ACATAGGATG GAATTGCTTT TCATTATTTT TCCAAAGCCG ACAGGTGCGC
1351 TGTGCTATGA ACATGGCAAT CGATAGAGAG AGGATATATCG AACAGTGCTT
1401 GGATGGCCAA GGCTATACGA TTAGTGGGCC TTTTGCTTCG AGTTCTCCTT
1451 CTTATAATAA ACAGATCGAA GGGTGGCATT ATTCTCCAGA AGAAGCAGCT
1501 CGTCTCCTGG AAGAAGAGGG ATGGATAGAT ACCGATGGCG ATGGAATCCG
55 1551 AGAAAAAGTT ATCGATGGTG TGATTGTCCC GTTCCGTTTC CGTTTATGCT
1601 ATTATGTAAA GAGTGTACCC GCTCATACCA TTGCAGATTA CGTAGCTACT
1651 GCTTGTAAAG AAATCGGAAT CGAGTGTAGC CTTCTAGGAC TAGATATGGC
1701 CGATCTTTCG CAAGCTTTTG ATGAAAGAA TTTTCGATGCT CTTTTAATGG
1751 GATGGTGTGTT AGGAATTCCT CCTGAGGATC CTAGGCTTTT ATGGCATTC

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```

1801 GAAGGGGCTA TGGAAAAGGG TTCAGCGAAT GTTGTAGGTT TCCATAATGA
1851 AGAAGCTGAT AAAATCATAG ACAGACTCAG CTACGAATAC GATCTGAAAG
1901 AACGTAATCG CCTGTACCAC CGTTTCCATG AAATTATTCA TGAGGAAGCT
1951 CCTTATGCTT TCTTGTTCCTC ACGACATTGT TCCTTACTTT ATAAGGATTA
2001 TGTAAGAAAT ATTTTCGTAC CTACACATAG AACAGATTTA ATTCCTGAAG
2051 CTCAGGATGA GACTGTCAAC GTAACATATG TATGGCTTGA GAAGAAGGAG
2101 GATCCGTGCT TAAGTACATC CTAA

```

The PSORT algorithm predicts inner membrane (0.162).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 189A) and also in GST/his form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 189B) and for FACS analysis.

These experiments show that cp6894 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 190

The following *C.pneumoniae* protein (PID 4377193) was identified in the 2D-PAGE experiment <SEQ ID 379; cp7193>:

```

1  MKRVITYKTIF CGLTLLTSL SCSLDPKGYN LETKNSRDLN QESVILKENR
51  ETPSLVKRLS RRSRLRFARR DQTQKDTLQV QANFKTYAEK ISEQDERDLS
101 FVVSSAAEKS SISLALSQGE IKDALYRIRE VHPLALIEAL AENPALIEGM
151 KMQQGRDWIW NLFLTQLSEV FSQAWSQGI SEEDIAAFAS TLGLDSGTVA
201 SIVQGERWPE LVDIVIT*

```

A predicted leader peptide is underlined.

The cp7193 nucleotide sequence <SEQ ID 380> is:

```

1  ATGAAAAGAG TCATTATATAA AACCATATTT TCGGGGTAA CTTTACTTAC
51  AAGTTTGAGT AGTTGTTCCC TGGATCCTAA AGGATATAAC CTAGAGACAA
101 AAAACTCGAG GGACTTAAAT CAAGAGTCTG TTATACTGAA GGAAAACCGT
151 GAAACACCTT CTCCTGTGTTAA GAGACTCTCT CGTCGTTCTC GAAGACTCTT
201 CGCTCGACGT GATCAAACTC AGAAGGATAC GCTGCAAGTG CAAGCTAACT
251 TTAAGACCTA CGCAGAAAAG ATTTTCAGAGC AGGACGAAAG AGACCTTTCT
301 TTCGTGTGCT CGTCTGCTGC AGAAAAGTCT TCAATTTCGT TAGCTTTGTC
351 TCAGGGTGAA ATTAAGGATG CTTTGTACCG TATCCGAGAA GTCCACCCCTC
401 TAGCTTTAAT AGAAGCTCTT GCTGAAAACC CTGCCTTGAT AGAAGGGATG
451 AAAAAGATGC AAGGCCGTGA TTGGATTGG AATCTTTTCT TAACACAATT
501 AAGTGAAGTA TTTTCTCAAG CTTGGTCTCA AGGGTTATC TCTGAAGAAG
551 ATATCGCCGC ATTTGCCTCC ACCTTAGGTT TGGACTCCGG GACCGTTGCG
601 TCCATTGTCC AAGGGGAAAG GTGGCCGAG CTTGTGGATA TAGTGATAAC
651 TTAA

```

The PSORT algorithm predicts periplasmic (0.925).

This shows that cp7193 is an immunoaccessible protein in the EB and that it is a useful immunogen.

These properties are not evident from the protein's sequence alone.

It will be appreciated that the invention has been described by way of example only and that modifications may be made whilst remaining within the spirit and scope of the invention.

TABLE II – sequences of the primers used to amplify Cpn genes.

Orf ID	N-terminus final primer	C-terminus final primer
CP0014P	GCGTC CCG GGT CATATG AAGTCTCTTTCCCA	GCGT CTC GAG ATGAAAGAGTTTTGCG
CP0015P	GCGTCCCGGGTCATATG TCAGCTCTGTTTTCTGA	GCGT CTC GAG GAATTTGGTATTTTGCTC
CP0016P	GCGTCCCGGGTCATATG GCCGATCTCACATTAG	GCGT CTC GAG GTCCAAGTTAAGGTAGCA
CP0017P	GCGT CCG GGT CATATG GGTATCAAGGGAAGT	GCGT CTC GAG AAATCCGAATCTTCC
CP0019P	GCGTCCCGGGTCAT ATGCAAGACTCTCAAGACTATAG	GCGT CTC GAG AAATCGGTATTTACCC
CP6260P	GCGTC CCG GGT GCTAGCACTACGATTCTTTAACCC	GCGT CTC GAG AAAACGAAATTTGCTTC
CP6397P	GCGTC CCG GGT CATATGTTTAACTGCTAAAAATCTATT	GCGT CTC GAG ATGAAAGAAGAGTCTCTCG
CP6456P	GCGTC CCG GGT CATATG TCATCTCTGTAAATAACA	GCGT CTC GAG CTGACCATCTCTCTGT
CP6466P	GCGTC CCG GGT CAT ATG TGCAAGGAGTCCAGT	GCGT CTC GAG ATTTTCCTTAGCATAAAG
CP6467P	GCGTC CCG GGT CAT ATG TGTTCCCATCCCAA	GCGT CTC GAG TAGTTTTCTATAAAACGAAAGTCT
CP6468P	GCGTC CCG GGT CAT ATG TGCTCTCTTACTCTCTC	GCGT CTC GAG GGGGAAATAGGTATATTTGA
CP6469P	GCGTC CCG GGT CAT ATG AGCTGCTCAAAGCAA	GCGT CTC GAG ACTTAAGATATCGATATTTTGA
CP6552P	GCGTC CCG GGT CAT ATG TGCCATAAGGAAGATG	GCGT CTC GAG ACCATTGTCTTGAGTCAT
CP6567P	GCGTC CCG GGT CAT ATG ACCTCACCAGTCCCC	GCGT CTC GAG AGAAGCCGGTAGAGGC
CP6576P	GCGTC CCG GGT CAT ATG ACTGAAAAAGTTAAAGAAGG	GCGT CTC GAG GAA CATGCCCCCTAA
CP6727P	GCGTC CCG GGT CATATGCTACATCCACTAATGGC	GCGT CTC GAG GAAAGAATAACGAGTTCC
CP6729P	GCGTC CCG GGT CAT ATGGCAGATGCTTCTTTATC	GCGT CTC GAG GAATGAATATCTTAGCC
CP6731P	GCGTC CCG GGT CATATGGCTGTGTGTGAATCAAT	GCGTC CAT GGC GGC CGC GAACTGGAACCTTACCTCC
CP6736P	GCGTC CCG GGT GCT AGCGTAGAAGTTATCATGCCCTT	GCGTC CAT GGC GGC CGC AAATCGTAATTTGCTTC
CP6737P	GCGT GGA TCC ATG GAGACTAGACTCGGAGG	GCGT CTC GAG AAATGTGATTTTAGTCC
CP6751P	GCGTC CCG GGT GCT AGC AATGAAGGTCTCCAAC	GCGT CTC GAG AAATCTCATTTACTCGC
CP6752P	GCGTGA ATT CAT ATGTTCCGGATGACFCTT	GCGT CTC GAG GAATTTTAAGGTACTTCTCTG
CP6753P	GCGTC CCG GGT GCT AGCACTCCCTACTCTCATAGAG	GCGT CTC GAG AAACCTTAAAGGTCTCTTC
CP6767P	GCGTC CCG GGT CAT ATG ATAAACAAATAGGCCGT	GCGT CTC GAG TTCGTAAGCAACTTCAGA
CP6828P	GCGTC CCG GGT CAT ATG AAGCAGATGCGTCTTT	GCGTC CAT GGC GGC CGC GAAACTAAGGAGAGGC
CP6830P	GCGTC CCG GGT CAT ATG GATCCCGCGTCTGTT	GCGTC CAT GGC GGC CGC GAATACAAACCGGATCC
CP6832P	GCGTC CCG GGT CAT ATG CATAAAGTAATAGTTTTCAATT	GCGT CTC GAG TAAACTAGAAAAAGTCTGC
CP6848P	GCGTC CCG GGT CAT ATG TCATCAAATCTACATCCC	GCGT CTC GAG AACCGAGCTATTTTAC
CP6849P	GCGTC CCG GGT GCT AGC AGCGGGGGTATAGAG	GCGT CTC GAG ATACACGTGGGTATTTTC
CP6850P	GCGTC CCG GGT CAT ATG TGCCGCAATGTAGAT	GCGT CTC GAG CTGTTTGCACTCTGCC
CP6854P	GCGTC CCG GGT GCT AGC TCAATAGCTATTGCAAG	GCGT CTC GAG TTATCGAAATGTCTTTG
CP6879P	GCGTC CCG GGT CAT ATG GCAACACCCGCTCAA	GCGTC CAT GGC GGC CGC TCCTTGAATTTGCTCTTGC
CP6894P	GCGTC CCG GGT CAT ATG TATAAAGATGTGTCTAGA	GCGT CTC GAG GGATGACTTAAGCAAG
CP6900P	GCGTC CCG GGT CAT ATG AAGATAAAATTTCTTGGAAAG	GCGT AAG CTT GGGAGACGATACCG
CP6952P	GCGTC CCG GGT CAT ATG CTCTCGGATCAATATATAGG	GCGT CTC GAG TCGAATTTCTTTTPTAGC
CP7034P	GCGTC CCG GGT CAT ATG AAAAACAGGTATATCAATG	GCGT AAG CTT AAACGCTGAAATTTATACC
CP7090P	GCGTC CCG GGT CAT ATG TGAGCCCTTCCCTT	GCGT CTC GAG GCGTGCATGAATCTTA
CP7091P	GCGTC CCG GGT CAT ATG GAAGAATTAGAAGTTGTGT	GCGT CTC GAG TAGTGTCTCTTTATCGGT
CP7170P	GCGTC CCG GGT CAT ATG CTAGGGGCTGGAAACC	GCGT AAG CTT AAATGCAAGCTGACG
CP7228P	GCGTC CCG GGT CAT ATG ACTGCTGTTCTTATCTTACA	GCGT CTC GAG ATCTGAAAGCGGAGG
CP7249P	GCGTC CCG GGT CAT ATG ATCCCATCCCTACC	GCGT CTC GAG ATCAGGTGTCTGAGACTT
CP7250P	GCGTC CCG GGT CAT ATG AATCTTTCAAACAGGTCT	GCGT CTC GAG ATTTTCTTAGAGAGACTCTC
CP0018P	GTGCGT CATATG GCAACCACTCCACTAA	ACTCGCTA GCGGCCGC TAATGAGGTCCCCAG
CP6270P	GTGCGT CATATG AATTATATAGGAGCTGCT	ACTCGCTA GCGGCCGC AAATTTGATTTTGCTACC
CP6735P	GTGCGT CATATG GCAGCACAAAGTTGTATAT	ACTCGCTA GCGGCCGC TGGCGTAGAAGTGATC
CP6998P	GTGCGT CATATG TTGCCTGTAGGGAAC	ACTCGCTA GCGGCCGC GAATCTGAACTGACCAGA
CP7033P	GTGCGT CATATG GTTAATCTTATTTGTCCA	ACTCGCTA GCGGCCGC TTGGAGATAACCAATATA
CP7287P	GTGCGT CATATG TTACACAGCTCAGAACTAGA	ACTCGCTA GCGGCCGC GAAAATAATACGGATACCA
CP0010P	GTGCGT CATATG GCAACTGCTGAAAATATA	GCGT CTCGAG GAATTGAACCTTACCC
CP0468P	GTGCGT GCTAGC ATTTTTATGACAACTCTAT	GCGT CTCGAG AAATGTCAATGACTCT
CP6272P	GTGCGT CATATG TTGACTCATCAAGAGGCT	GCGT CTCGAG GAAGGGAGGTTTTTTAGGT
CP6273P	GTGCGT CATATG ACATATCTGGAAGCTC	ACTCGCTA GCGGCCGC CTCCACAATTTTATG
CP6362P	GTGCGT CATATG CCCTTTGATATTAATTTATATA	GCGT CTCGAG TCGTTTCCAAATCCA
CP6372P	GTGCGT CATATG AAACAACACTATTTCTAAATA	GCGT CTCGAG TTCTTTGTGTTTTTCT
CP6390P	GTGCGT CATATG CGAGAGGTGCTTAAG	ACTCGCTA GCGGCCGC TCTCTAGACAGCCTT
CP6402P	GTGCGT CATATG AATGTTGCGGATCTCCCTT	GCGT CTCGAG GAAGGGGTGCGCCGT
CP6446P	GTGCGT CATATG TGTAAATCAAAAGCCCTCTT	GCGT CTCGAG GGGCTGAGGAGGAAC
CP6520P	GTGCGT GCTAGC AAACACTACCTATCATTTCTT	GCGT CTCGAG CAGAAAGGCTTTTCTTT
CP6577P	GTGCGT CATATG AATTAGGCTATGTTAATTTA	GCGT CTCGAG GTTTTGTGTTTTTGAAGA
CP6602P	GTGCGT CATATG GCAGCATCAGGAGGCA	GCGT CTCGAG TGACCAAGGATAGGGTTTAG

CP6607P	GTGCGT CATATG CCTCGTGGTGACACTTT	GCGT CTCGAG CGCTGCTTCTTGCTC
CP6615P	GTGCGT CATATG TGCTCTCAAAAAACGACAA	GCGT CTCGAG TGAAGAGGCGCCATC
CP6624P	GTGCGT CATATG GATGCGAAAATGGGA	GCGT CTCGAG TCTTTGACATTCAAGAGC
CP6672P	GTGCGT CATATG ATTCTTACCATTGTTAATG	GCGT CTCGAG GTCATACAATTTCTTATATA
CP6679P	GTGCGT CATATG TGCACTCACTTAGGCT	GCGT CTCGAG CGAGTAGTTAGCACAAAC
CP6717P	GTGCGT GCTAGC AAGACAATCGTAGCTTCA	ACTCGCTA GCGGCCGC GGCTGGCATATAGGT
CP6784P	GTGCGT GCTAGC AAATCAAGATGTTCTATTGATA	GCGT CTCGAG TCCAAAACAACCCCT
CP6802P	GTGCGT CATATG TCGTAAGTTATATTATTCCTT	GCGT CTCGAG CAGTCGGGCTTGTTG
CP6847P	GTGCGT CATATG TCGGATCTTTTACGAG	GCGT CTCGAG TTTTCTACACTGTGTATAAATA
CP6884P	GTGCGT CATATG AATCAGCTGCTTCT	GCGT CTCGAG AGAGAAGGTAATGTACC
CP6886P	GTGCGT CATATG TGTCTACTTATTATCTATCTCTAC	GCGT CTCGAG TTCAGAAAAATGGCT
CP6890P	GTGCGT CATATG TCCCCACGACGACAA	GCGT CTCGAG TCTTCGACATTTAGC
CP6960P	GTGCGT CATATG TGTGACGTACGGTCTA	ACTCGCTA GCGGCCGC TTCACCTTGATTTCCT
CP6968P	GTGCGT CATATG TCGGATGCAAAAC	ACTCGCTA GCGGCCGC GGAAGTATGCTTAGATATT
CP6969P	GTGCGT CATATG TGCTGTGGTTACTCTATT	ACTCGCTA GCGGCCGC AAAAAGGTCATAGTATACCT
CP7005P	GTGCGT CATATG AAAACTGTGATATTGAACA	GCGT CTCGAG CTGAGCTTCTATTTCATTAT
CP7072P	GTGCGT CATATG CCCATTTATGGGAAA	GCGT CTCGAG GTTGAGCAAAGGTTTG
CP7101P	GTGCGT CATATG TATTCGTGTACAGCAA	GCGT CTCGAG GAAAAATTTCTTAGGGAG
CP7102P	GTGCGT CATATG GCGCTAAAGCAAAT	GCGT CTCGAG TGAAAAATGAAAGGATGGT
CP7105P	GTGCGT GCTAGC AGTCTATATCAAAAATGGTG	GCGT CTCGAG ATCTTTTATTTGGTTATCT
CP7106P	GTGCGT CATATG AAAGATTTGGGGACTCT	GCGT CTCGAG GAATCCTAAGGCATACCTA
CP7107P	GTGCGT GCTAGC AGTATAGTCAGAAATTCGCA	GCGT CTCGAG GAAGCTAAGATTATAGCTACTTT
CP7108P	GTGCGT GCTAGC GCGGCCCTTTTCCA	ACTCGCTA GCGGCCGC TTTATGTATATGGAACAGATAGG
CP7109P	GTGCGT CATATG GGACATTTTATTTGATATTG	ACTCGCTA GCGGCCGC ATCATCAAGGTAGATAAAG
CP7110P	GTGCGT CATATG GGTATTTGCTATGTAAATTACA	GCGT CTCGAG TTCTGATTTGGACTCCA
CP7127P	GTGCGT CATATG GTGGCTTTAAGGATAGC	ACTCGCTA GCGGCCGC GCAGCCATCGTATTC
CP7130P	GTGCGT CATATG TTCAATATGCGAGG	GCGT CTCGAG CTCTTTATTGAACTTTG
CP7140P	GTGCGT CATATG ACAGCCGGAGCAGCT	GCGT CTCGAG AGCACCCCTCAATTTTATTG
CP7182P	GTGCGT CATATG GGATATGTTTCTATGTGATC	GCGT CTCGAG GCTACTAAATCGAATCGA
CP6262P	GTGCGT CATATG ATCCCTGGATTAAAGTTCA	ACTCGCTA GCGGCCGC TTCCTGGGAGCTTGA
CP6266P	GTGCGT CATATG TACCAGGAGAACTAAGAT	ACTCGCTA GCGGCCGC GATTTCCTTCTTCAGCTC
CP6296P	GTGCGT CATATG GAGGAGGTGTCTGAGTAT	ACTCGCTA GCGGCCGC ATGTTTCTTTTACTCTTTCT
CP6419P	GTGCGT CATATG GCTCCAGTCCGTGTT	GCGT CTCGAG AAGTGTTCGTTCGAAAT
CP6601P	GTGCGT CATATG AATAAGCTACTCAATTTCTGT	GCGT CTCGAG GAAAAATCTGAATTTCTTCT
CP6639P	GTGCGT CATATG TTAATAATCAAGCAATTCA	GCGT CTCGAG AGGAACTAAAACTCATCT
CP6664P	GTGCGT GCTAGC GTTTTATTTTCAATGCTCAA	ACTCGCTA GCGGCCGC CTTAGAAAGACTATTTTCTAAGTA
CP6696P	GTGCGT CATATG TGCCTGATAAATGGG	GCGT CTCGAG ATTCACTCTTCTGTAAGAAAT
CP6757P	GTGCGT CATATG GCAGTTGGTGGCGT	ACTCGCTA GCGGCCGC CTGTCCCTCTGAGAC
CP6790P	GTGCGT GCTAGC AGTGAACACAAAAATCA	ACTCGCTA GCGGCCGC CTATCTGTCTGTATCAATA
CP6814P	GTGCGT CATATG CATGACGCACTTCTAAG	GCGT CTCGAG TACAGCTGCGCGA
CP6834P	GTGCGT CATATG GTTATGGGAACCTATATCG	GCGT CTCGAG TACATTGTATTTGATTTCAG
CP6878P	GTGCGT CATATG AACGTCCCTGATTC	GCGT CTCGAG GCTAGCGGCTCTTTC
CP6892P	GTGCGT CATATG CAGAAGCATCCTTCCT	ACTCGCTA GCGGCCGC TCCTCTTTAGGAAATGG
CP6909P	GTGCGT CATATG TCTCTTTAGGAAATGG	GCGT CTCGAG CAGTGCCTAAGTAGGGA
CP7015P	GTGCGT CATATG GCAGTACGATTAATTTGTTG	GCGT CTCGAG TTTATTTGATGCTATTTTATATTTTC
CP7035P	GTGCGT GCTAGC AGCAGAAAAAGACAATGA	GCGT CTCGAG ATTTTGAGTGTCTTGCA
CP7073P	GTGCGT CATATG ATTACCATAAATCAGTG	GCGT CTCGAG TATCCATCGACTTATAGC
CP7085P	GTGCGT GCTAGC TGTATTTTCCCTTACGTA	ACTCGCTA GCGGCCGC GGATTTCTGCATACTCTG
CP7092P	GTGCGT CATATG TCTCTCTTCTTAAAAAA	GCGT CTCGAG GGATTCATTACTGACCA
CP7093P	GTGCGT CATATG AAATACCGCTTACG	GCGT CTCGAG ATTCTGTAGGCTACGT
CP7094P	GTGCGT CATATG GTACACTTCTCTCATAAACC	GCGT CTCGAG TAAAGTTGTATTTGCGGTAT
CP7132P	GTGCGT CATATG TTGTTATTAGGGACTTTAGGA	GCGT CTCGAG TTTCCCAACCGCA
CP7133P	GTGCGT CATATG GCTGCGAATGCTC	GCGT CTCGAG TAAATTAATACTCTTTGAAGG
CP7177P	GTGCGT CATATG CCTACTCAAGTTAAAACAGA	GCGT CTCGAG AAGTTTATATTTCAGCACTT
CP7184P	GTGCGT GCTAGC CATATAGGATTTTGCCA	GCGT CTCGAG GTACTTAGCAAAGCGAT
CP7206P	GTGCGT GCTAGC AAGAAGCTATATCACCTTA	GCGT CTCGAG CACACCGAGGAAAC
CP7222P	GTGCGT CATATG GTAGTTTCAGAGAAAAAGTC	GCGT CTCGAG ACGTATGCGCAACTG
CP7223P	GTGCGT CATATG GAAGTATTAGACCGCTCT	GCGT CTCGAG CGAGAAAAAGCTTCC
CP7224P	GTGCGT CATATG ATGAAGAAAAATTCGAAA	ACTCGCTA GCGGCCGC TAAGCATTCACAAATGA
CP7225P	GTGCGT CATATG CATATTTTGCTTGATCGT	GCGT CTCGAG TCTTTTAACTAATCTTGTTCTT
CP7303P	GTGCGT CATATG CTGTCTTATTTGTTTGATCC	GCGT CTCGAG AAAATATACGGAACCTCG
CP7304P	GTGCGT GCTAGC GAAGTTTATAGTTTTCCTC	GCGT CTCGAG TTTTGTATCTCTTAAGAAG
CP7305P	GTGCGT CATATG GAAGTTTATAGTTTTCACCTT	GCGT CTCGAG ACTCCTTGAGAGGGAA
CP7307P	GTGCGT CATATG CTAAATCATGCTAAAAAGC	ACTCGCTA GCGGCCGC CTCTTTTATTTTAGGAAGCT

CP7342P	GTGCGT CATATG AAAAAAAAAATTATTTCTACT	ACTCGCTA GCGGCCGC CACACTCTGTTCTCTG
CP7347P	GTGCGT CATATG TTTTCTAAGGATTGACTAA	GCGT CTCGAG CGAAGCAGAAGTCGT
CP7353P	GTGCGT CATATG AATATGCGCTGTTCTCTCT	GCGT CTCGAG GGGGCGTAGGTTGTA
CP7193P	GTGCGT CATATG TGTTCCTGGAATCCT	ACTCGCTA GCGGCCGC AGTTATCACTATATCCACAAG
CP7248P	GTGCGT GCTAGC CTTGAACATTCTAAACAAGAT	GCGT CTCGAG ACGTAGTTTAAGAGCAGACT
CP7261P	GTGCGT CATATG TGTCTATCTGCCTACATAG	GCGT CTCGAG TTTTGATGCTTCTTTCA
CP7280P	GTGCGT CATATG GACCAGAAAATTGAAAA	GCGT CTCGAG AGAGGCTTCTGAGTGC
CP7302P	GTGCGT CATATG AATTTCATTGTAGTGTAGT	GCGT CTCGAG GAACAGTTGATTTGTG
CP7306P	GTGCGT CATATG CTTCTTTATCAGGGCA	ACTCGCTA GCGGCCGC TTCTTCAGGTTTCAGG
CP7367P	GTGCGT GCTAGC CGTTATGCCGAGGTC	GCGT CTCGAG TTCGTGCAATTGCTG
CP7408P	GTGCGT CATATG TTGAAAATCCAGAAAAA	GCGT CTCGAG ATTCAATTTTCGGAAGAG
CP7409P	GTGCGT CATATG AGACGTTATCTTTTCATGGT	GCGT CTCGAG CCCTTGTCTCTTACATAG
CP8733P	GTGCGT ACTAGT TGTCACTACAGTCACTAG	GCGT CTCGAG GAATCGGAGTTTGTA
CP8728P	GTGCGT ACTAGT AAGTCCTCTGTCTCTTGG	GCGT CTCGAG GAAACAAAACCTTAGAGCCC

TABLE III – Proteins with best results in FACS analysis

cp number	Molecular Weight (kDa)		Fusion type
	Theoretical	Western Blot	
6260	97.5	94; 70	GST
6270	87.5	-	GST
6272	78.0	90	GST
6273	58.6	74; 64; 50	GST
6296	31.1	-	GST
6390	88.9	102	GST
6456	42.5	89; 67; 45	GST
6466	57.5	59; 56	His
6467	59.0	67	GST
6552	28.4	50; 27	GST
6576	86.0	79; 70; 62; 45	GST
6577	17.3	12	GST
6602	43.4	53; 42; 34	GST
6664	54.5	104; 45	GST
6696	47.9	95; 53	GST
6727	130.0-142.9	123; 61; 39	His
6729	94.8	multiple bands	GST
6731	95.5	97	GST
6733	97.1	104	His
6736	100.1	98; 93; 66; 60	GST
6737	101.2	multiple bands	GST
6751	100.2	95; 71	GST
6752	102.1	97; 48	His
6767	29.1	28	GST
6784	32.9	35	GST
6790	71.3	multiple bands	His
6802	29.7	-	GST
6814	29.6	28	GST

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6830	177.4	174; 91; 13	GST
6849	57.3	multiple bands	GST
6850	7.4-9.4	61; 14; 8	GST
6854	42.2	-	GST
6878	40.4	-	GST
6900	28.0	-	GST
6960	25.6	75; 35	GST
6968	34.6	83; 53; 35	GST
6998	39.3	multiple bands	GST
7033	68.2	multiple bands	GST
7101	113	105	GST
7102	63.4	-	GST
7105	29.2	30	GST
7106	39.5	72;46	GST
7107	71.4	67; 31	His
7108	35.9	35	GST
7111	46.1	51	GST
7132	17.9	57; 47; 17	His
7140	36.2-29.8	50; 38; 34	GST
7170	34.4	77; 33	GST
7224	39.4	40	GST
7287	167.3	180	GST
7306	50.1	50	GST

TABLE IV – FACS-positive proteins not found in *C.trachomatis*

cp7105	cp6390
cp7106	cp6784
cp7107	cp6296
cp7108	

TABLE V – Proteins identified by MALDI-TOF following 2D electrophoresis

cp6270	cp6733	cp6900
cp6552	cp6736	cp6960
cp6576	cp6737	cp6998
cp6577	cp6752	cp7033
cp6602	cp6767	cp7108
cp6664	cp6784	cp7111
cp6727	cp6790	cp7170
cp6728	cp6830	cp7287
cp6729	cp6849	cp7306

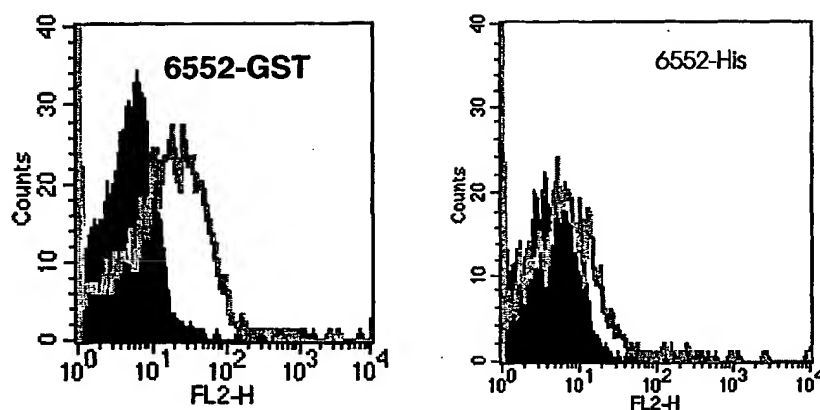
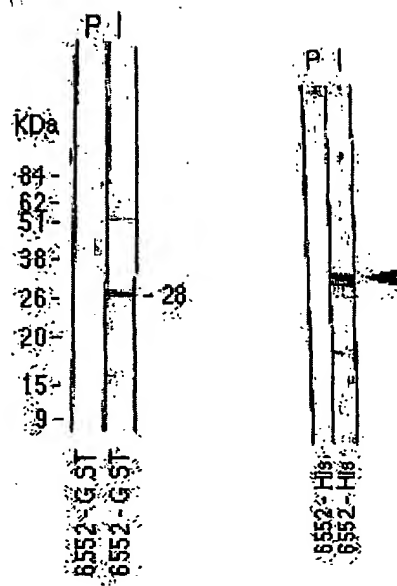
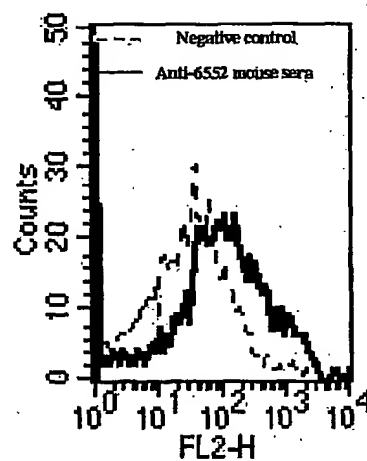
CLAIMS

1. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 97, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, & 377.
2. A protein having 50% or greater sequence identity to a protein according to claim 1.
3. A protein comprising a fragment of an amino acid sequence selected from the group consisting of SEQ IDs 97, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, & 377.
4. A nucleic acid molecule which encodes a protein according to any one of claims 1 to 3.
5. A nucleic acid molecule according to claim 4, comprising a nucleotide sequence selected from the group consisting of SEQ IDs 98, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318,

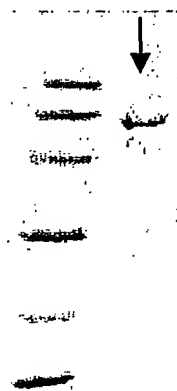
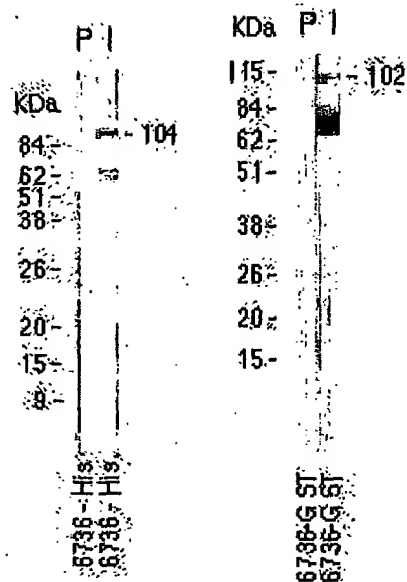
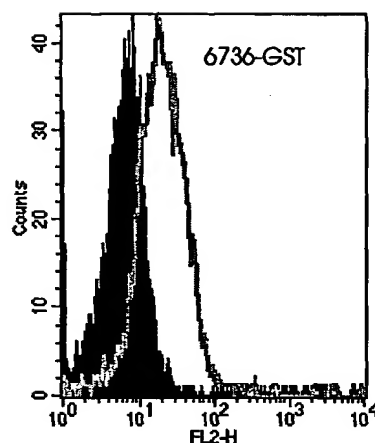
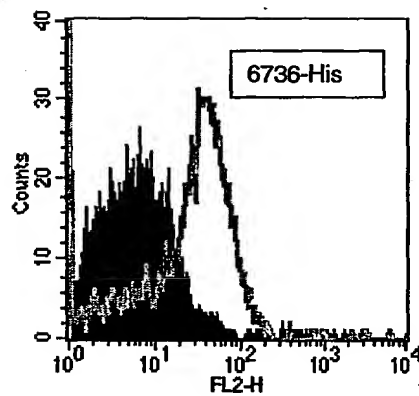
320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, & 378.

- 5 6. A nucleic acid molecule comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ IDs 98, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 10 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, & 378.
- 15 7. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to any one of claims 4 to 6.
8. A nucleic acid molecule comprising a nucleotide sequences having 50% or greater sequence identity to a nucleic acid molecule according to any one of claims 4 to 7.
9. A nucleic acid molecule which can hybridise to a nucleic acid molecule according to any one of claims 4 to 8 under high stringency conditions.
- 20 10. A composition comprising a protein or a nucleic acid molecule according to any preceding claim.
11. A composition according to claim 10 being a vaccine composition.
12. A composition according to claim 10 or claim 11 for use as a pharmaceutical.
- 25 13. The use of a composition according to claim 10 in the manufacture of a medicament for the treatment or prevention of infection due to *Chlamydia* bacteria, particularly *Chlamydia pneumoniae*.

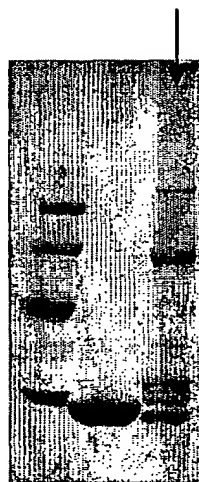
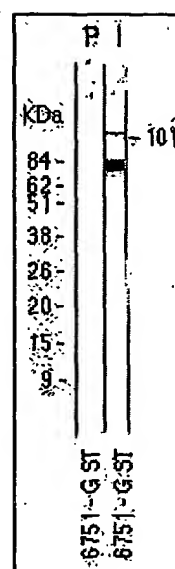
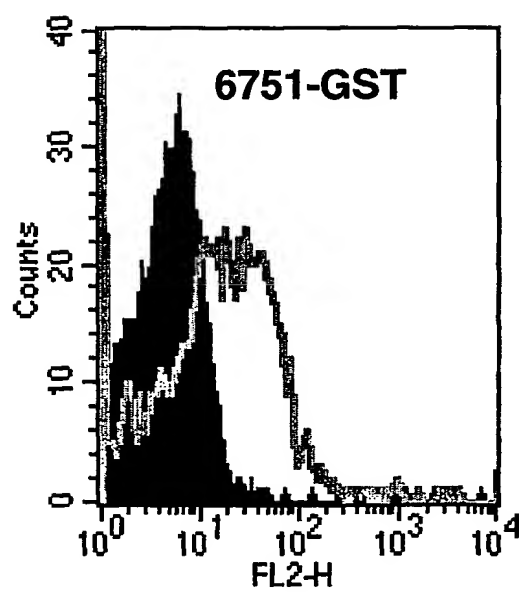
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FIGURE 1**Fig. 1A****Fig. 1B****Fig. 1C**

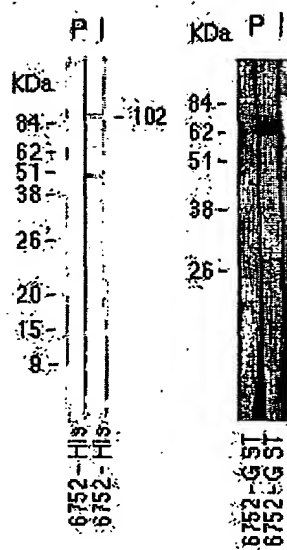
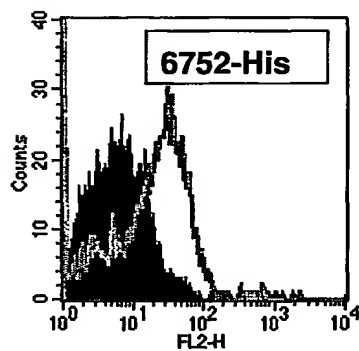
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FIGURE 2**Fig. 2A****Fig. 2B****Fig. 2C**

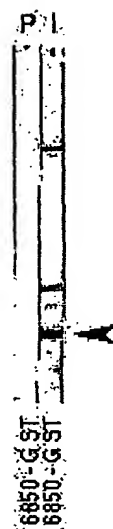
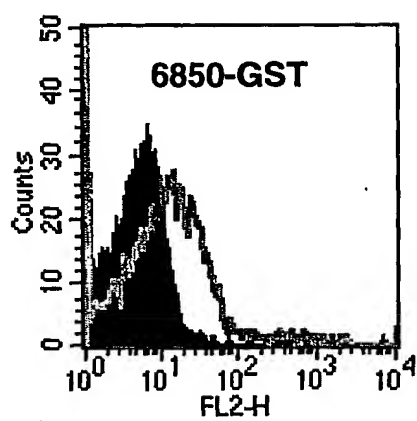
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FIGURE 3**FIG. 3A****FIG. 3B****FIG. 3C**

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FIGURE 4**FIG. 4A****FIG. 4B****FIG. 4C**

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FIGURE 5**Fig. 5A****Fig. 5B****Fig. 5C**

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FIGURE 6

FIG. 6A

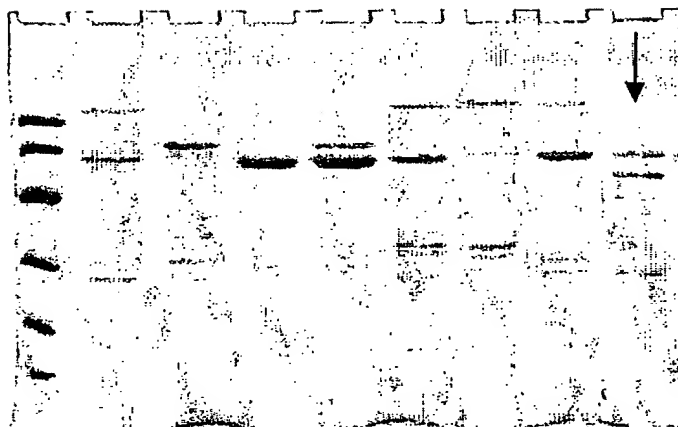


FIG. 6B

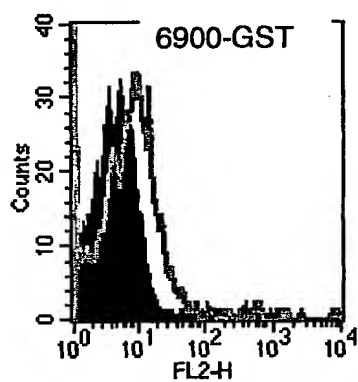
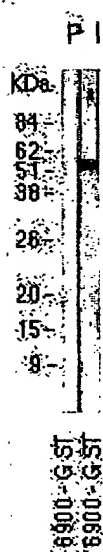


FIG. 6C



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FIGURE 7

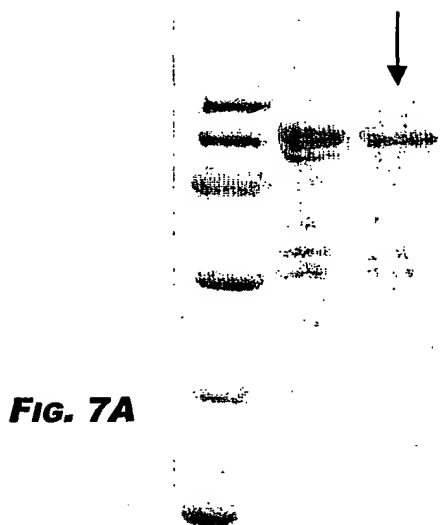


FIG. 7A

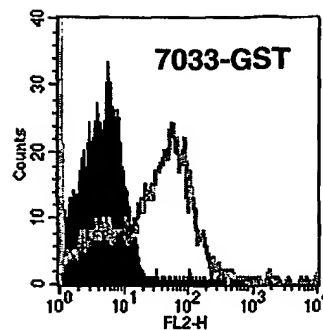


FIG. 7B

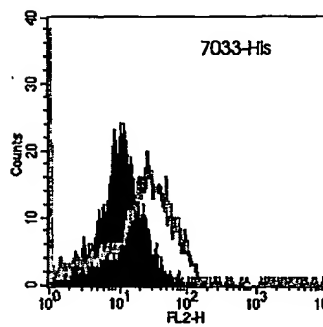
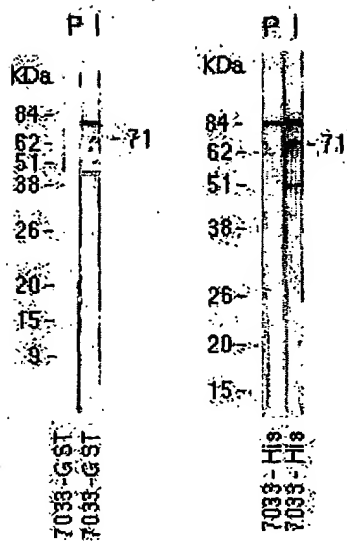


FIG. 7c



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FIGURE 8

Fig. 8A

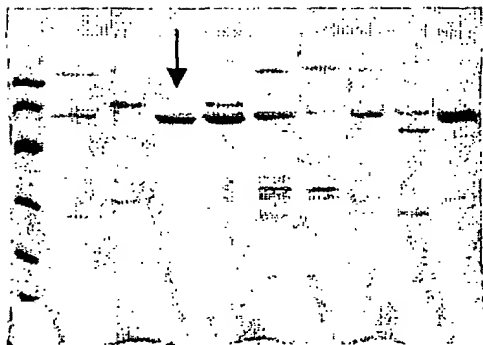
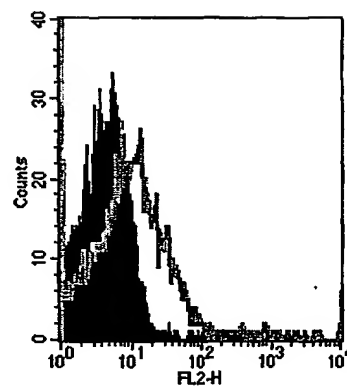


Fig. 8B



Fig. 8C



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FIGURE 9

FIG. 9A

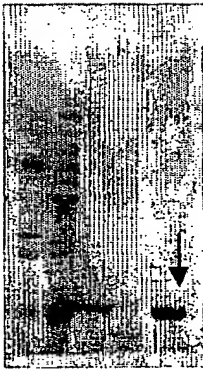


FIG. 9B

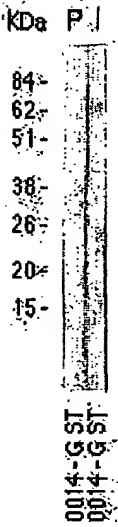
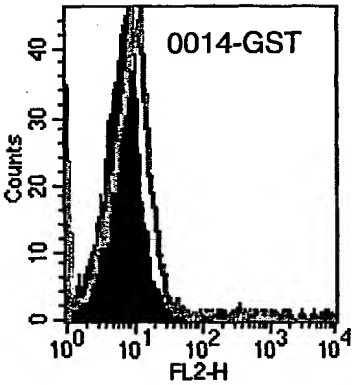


FIG. 9C



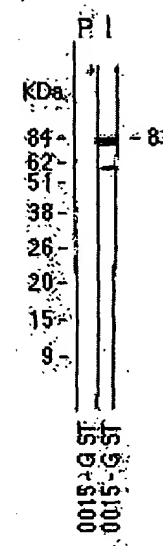
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FIGURE 10

FIG. 10A



FIG. 10B



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FIGURE 11

Fig. 11A

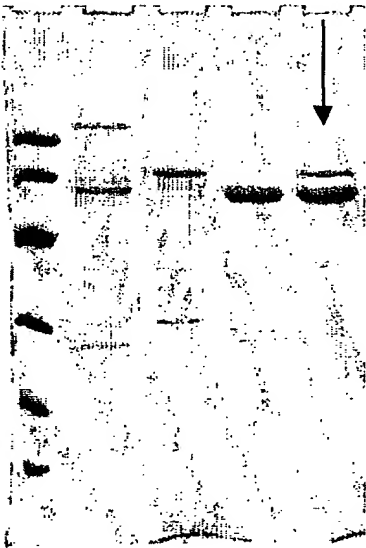
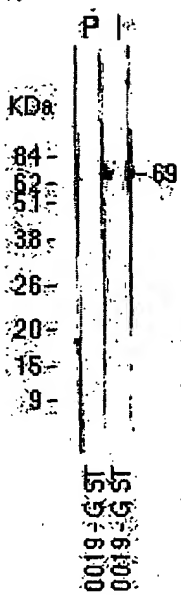


Fig. 11B



Fig. 11C



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FIGURE 12

FIG. 12A

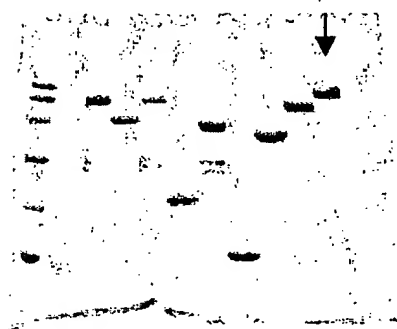


FIG. 12B

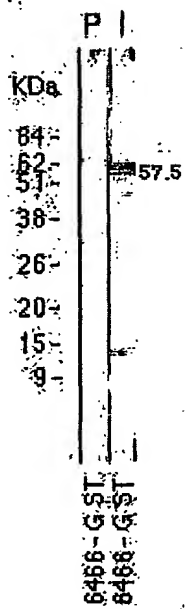
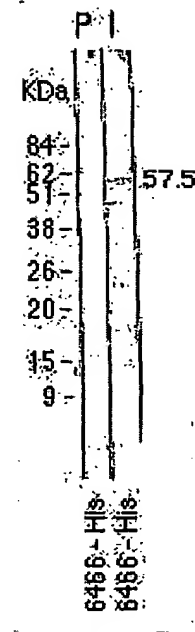
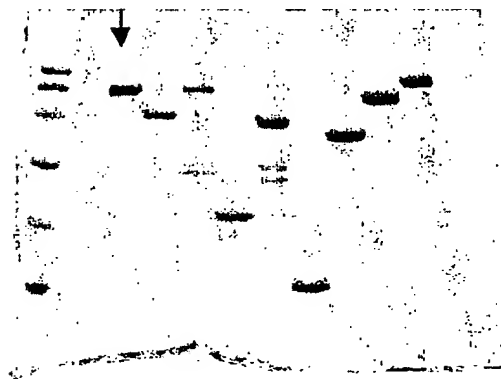
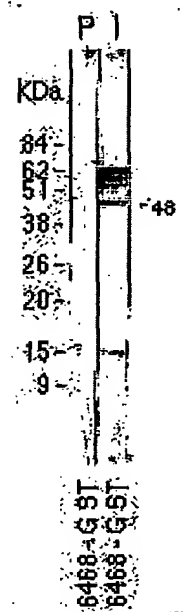


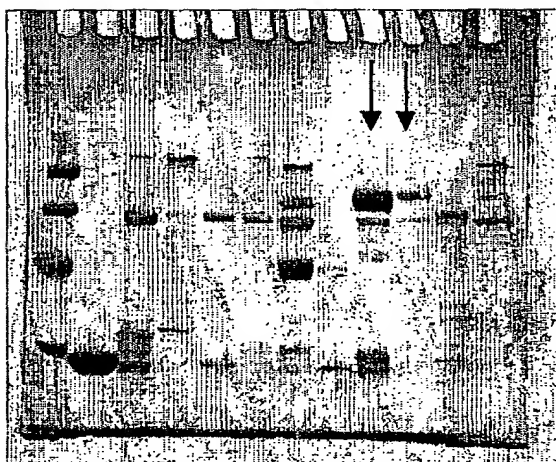
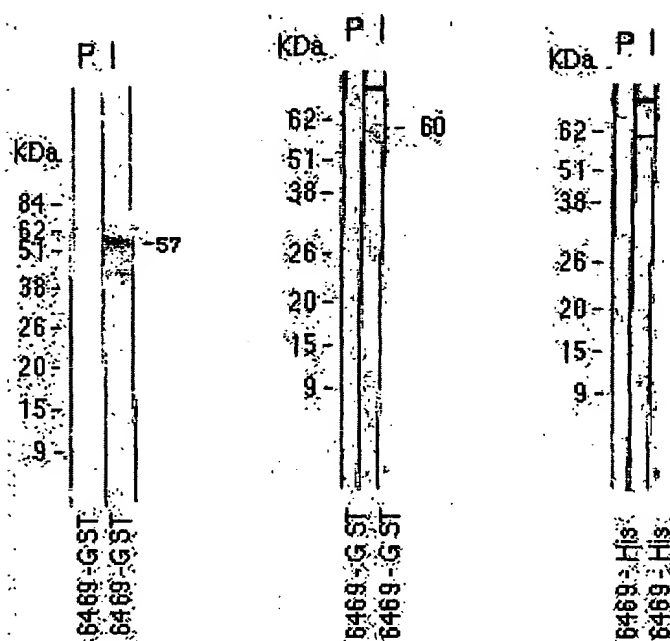
FIG. 12C



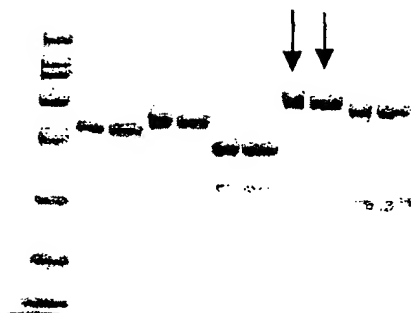
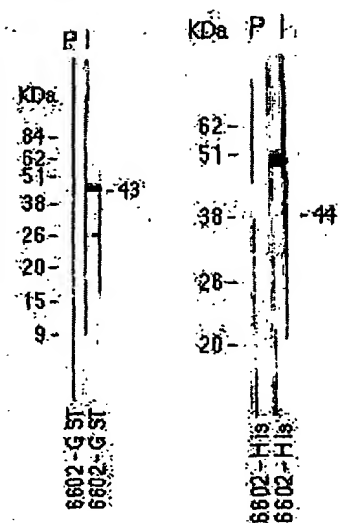
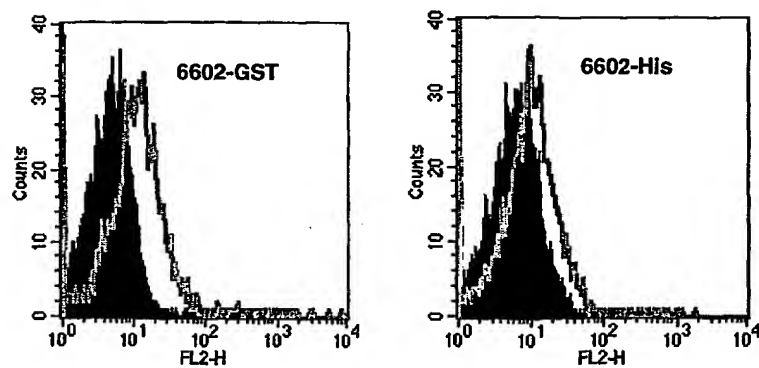
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FIGURE 13**Fig. 13A****Fig. 13B**

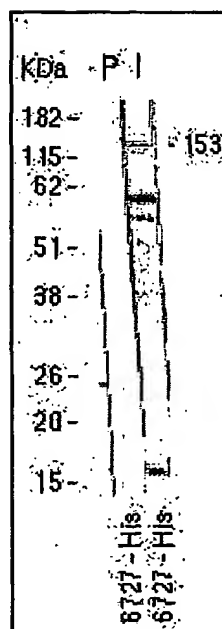
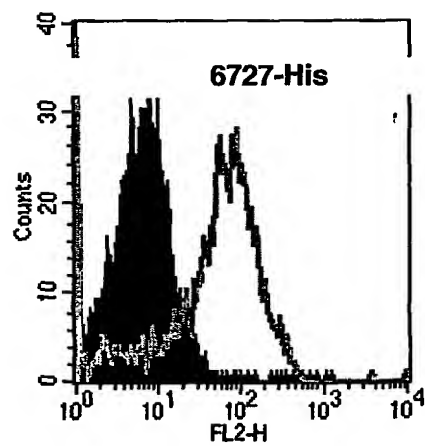
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FIGURE 14**FIG. 14A****FIG. 14B**

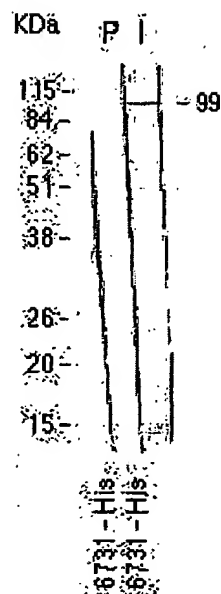
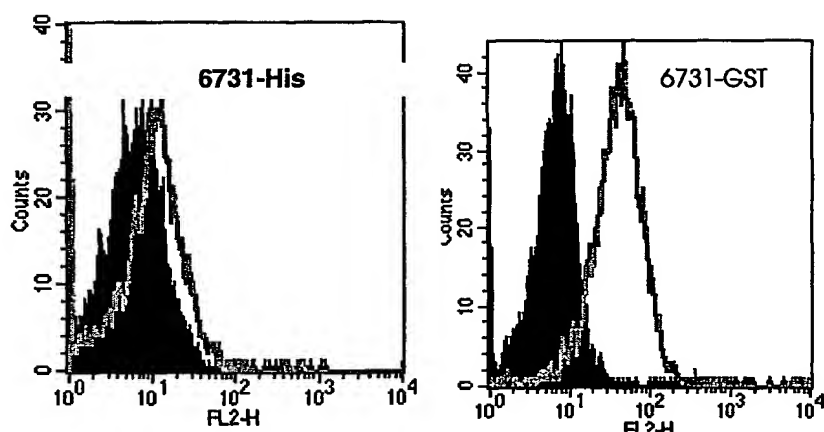
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FIGURE 15**Fig. 15A****Fig. 15B****Fig. 15C**

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FIGURE 16**Fig. 16A****Fig. 16B****Fig. 16C**

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FIGURE 17**FIG. 17A****FIG. 17B****FIG. 17C**

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FIGURE 18

Fig. 18A

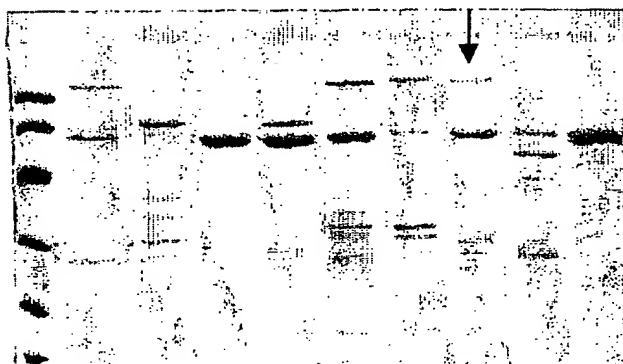


Fig. 18B

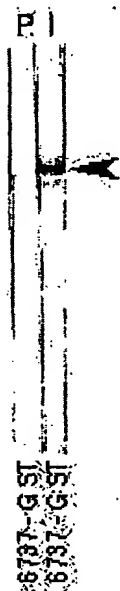
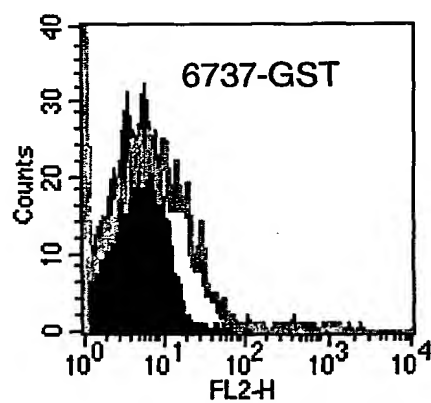


Fig. 18C



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FIGURE 19

Fig. 19A

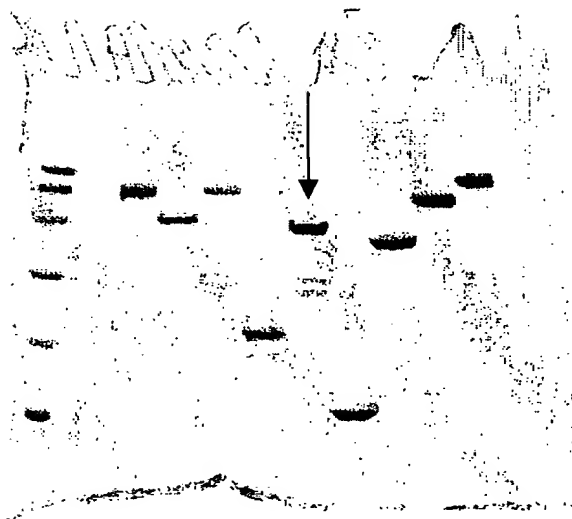
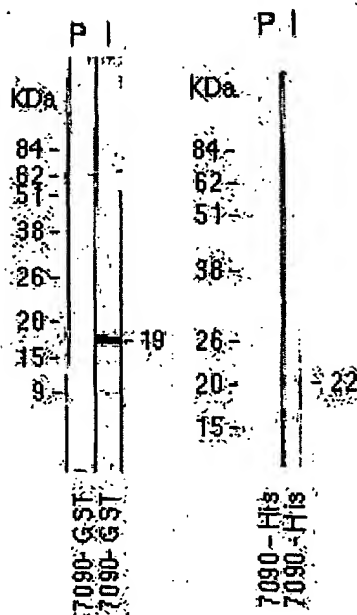


Fig. 19B



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FIGURE 20

Fig. 20A

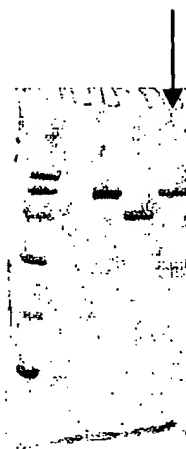
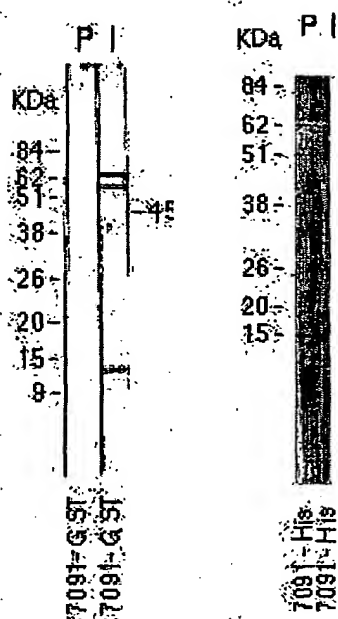


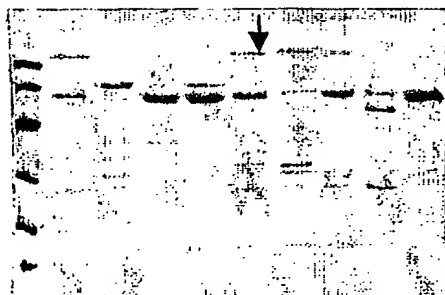
Fig. 20B



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FIGURE 21

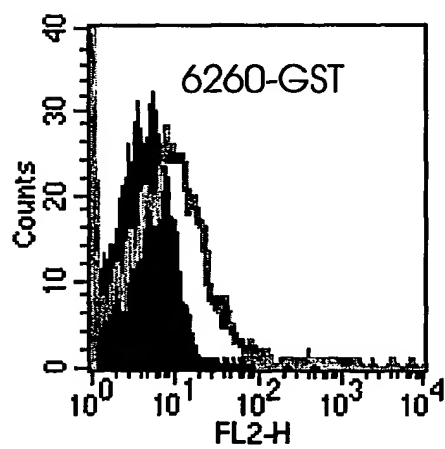
**FIG.
21A**



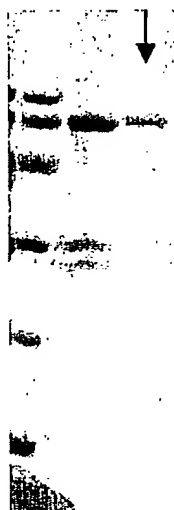
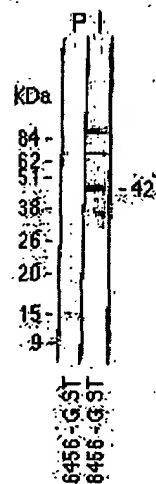
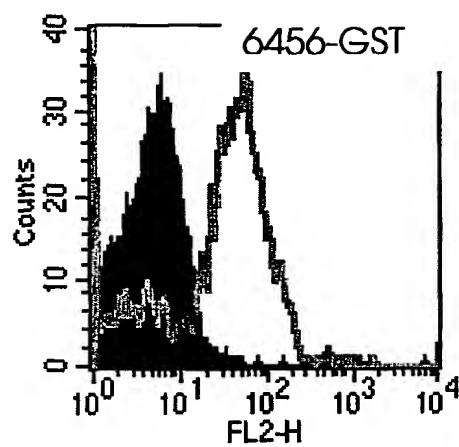
**FIG.
21B**



**FIG.
21C**



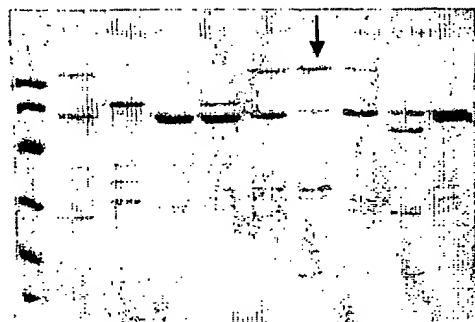
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FIGURE 22**FIG.
22A****FIG.
22B****FIG.
22C**

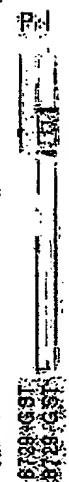
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FIGURE 23

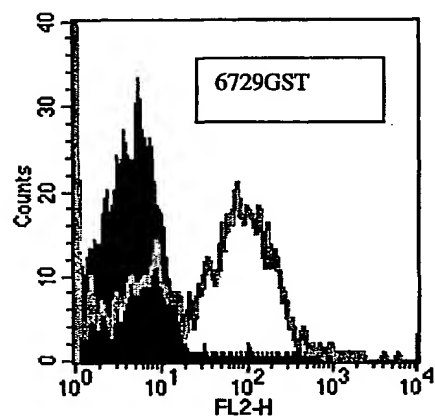
**FIG.
23A**



**FIG.
23B**



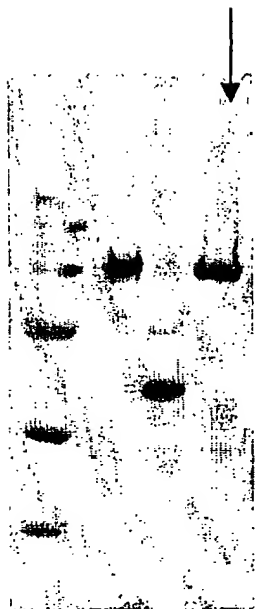
**FIG.
23C**



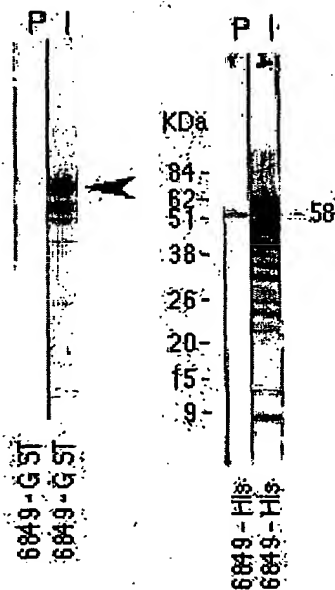
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FIGURE 24

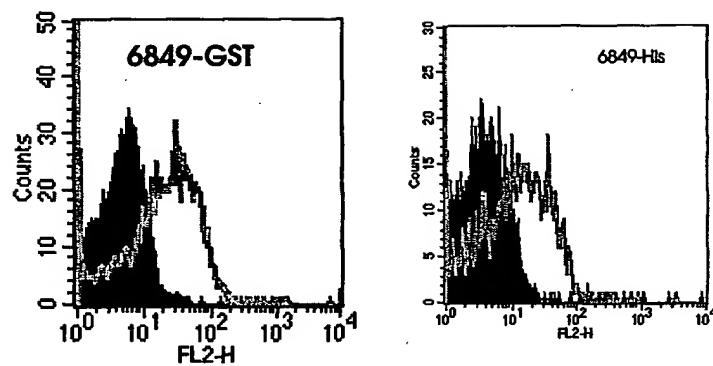
**FIG.
24A**



**FIG.
24B**



**FIG.
24C**



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FIGURE 25

FIG. 25A

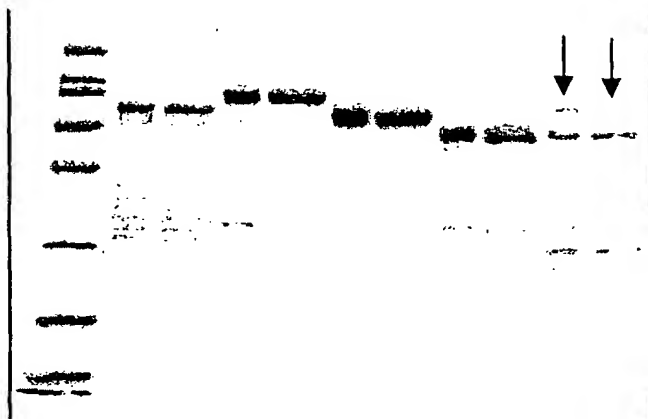


FIG. 25C

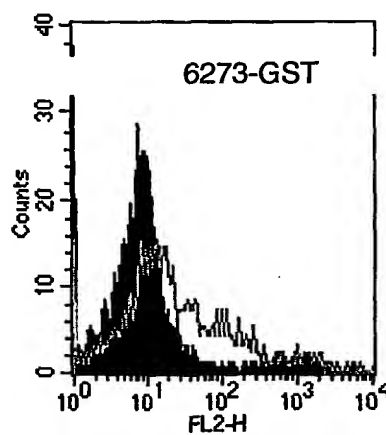
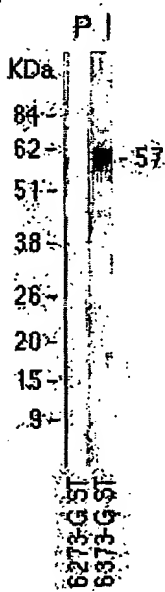


FIG. 25B



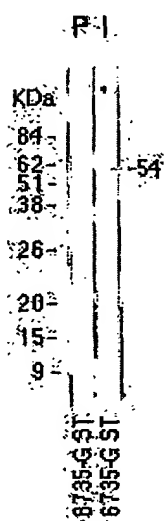
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FIGURE 26

FIG. 26A



FIG. 26B



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FIGURE 27

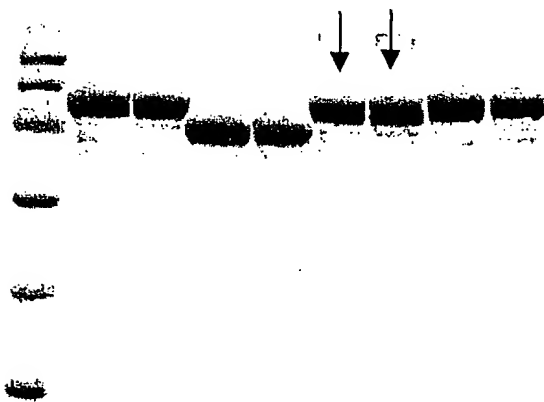


Fig. 27A

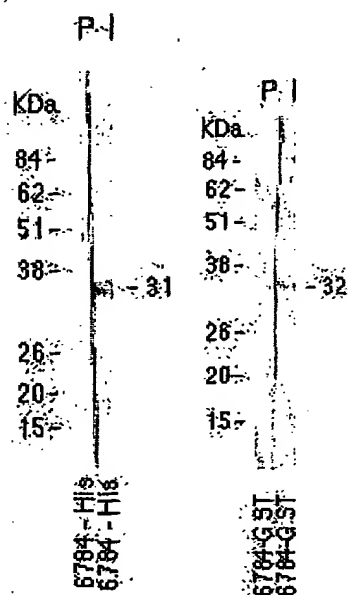


Fig. 27B

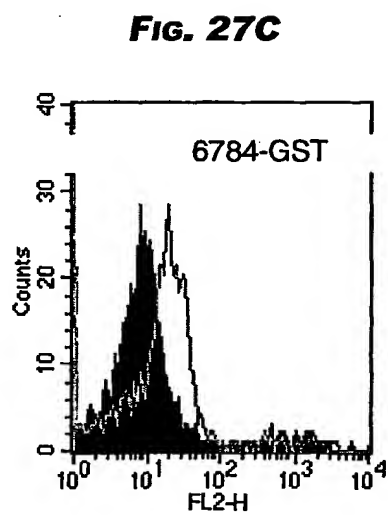


FIG. 27C

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FIGURE 28

FIG. 28A

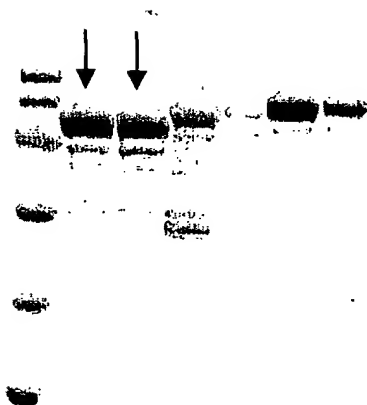


FIG. 28B

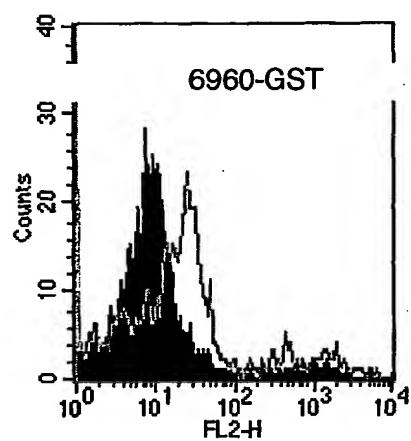
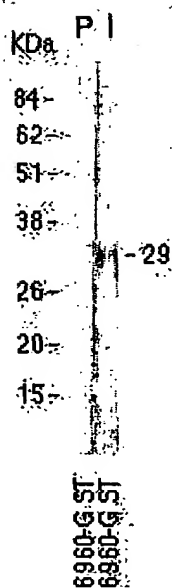
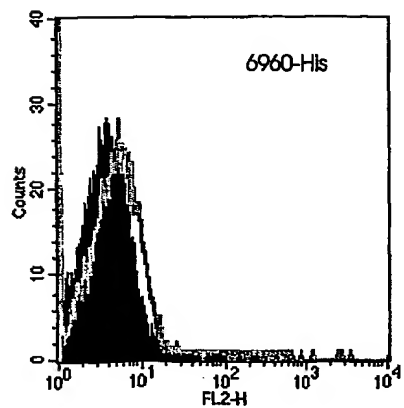


FIG. 28C



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FIGURE 29

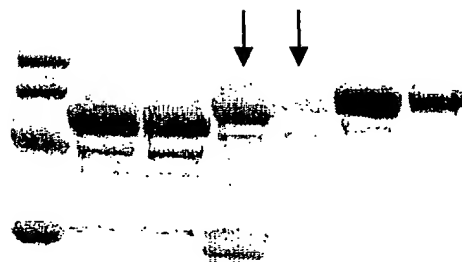


Fig. 29A

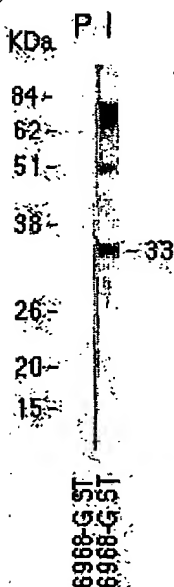
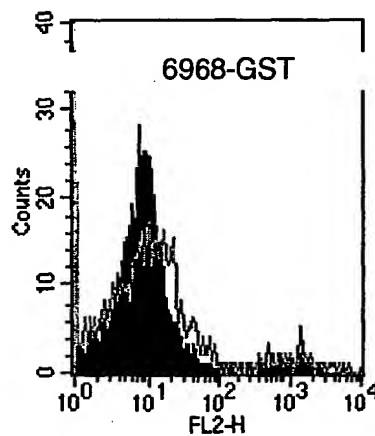


Fig. 29B

Fig. 29C



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FIGURE 30

Fig. 30A

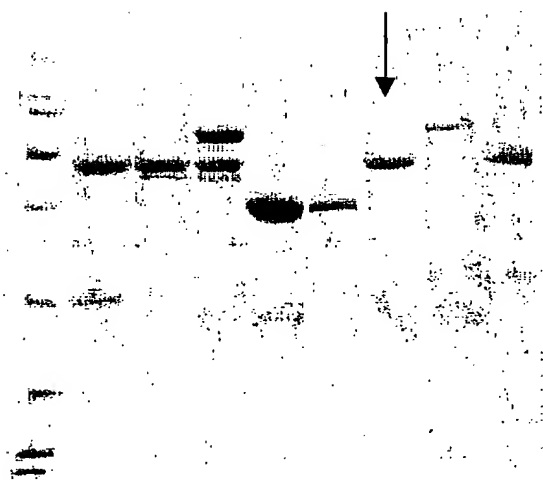


Fig. 30B

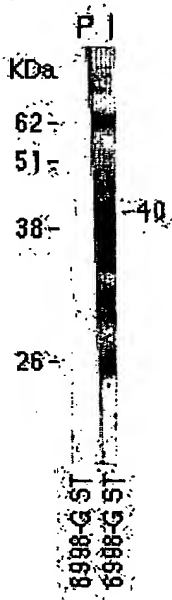
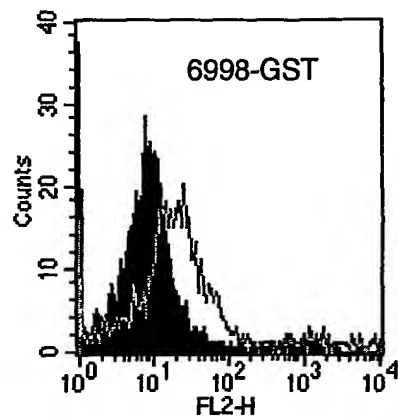
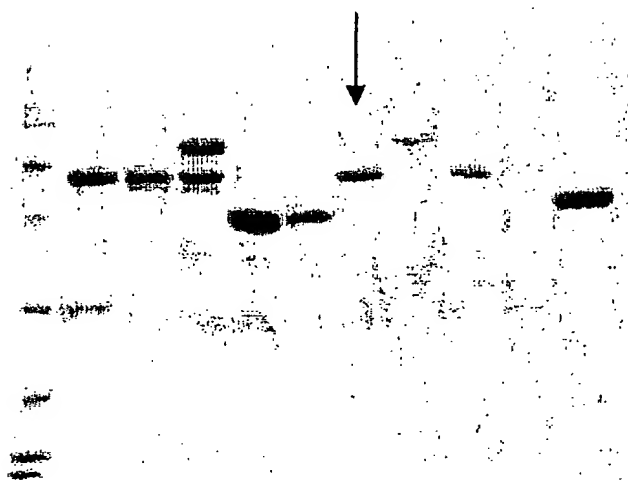
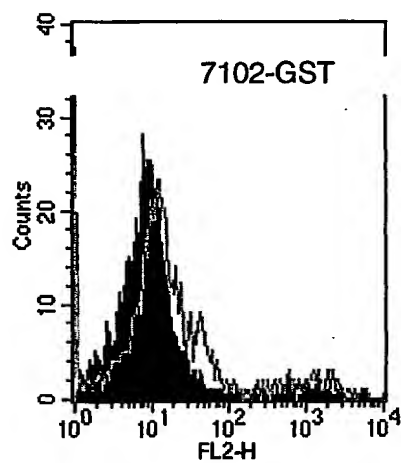


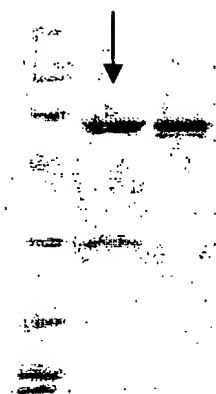
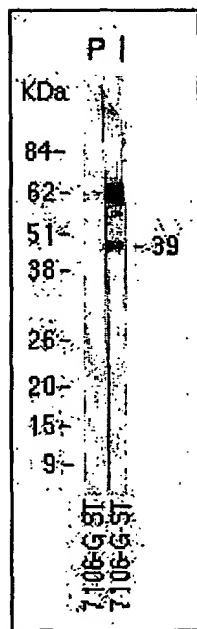
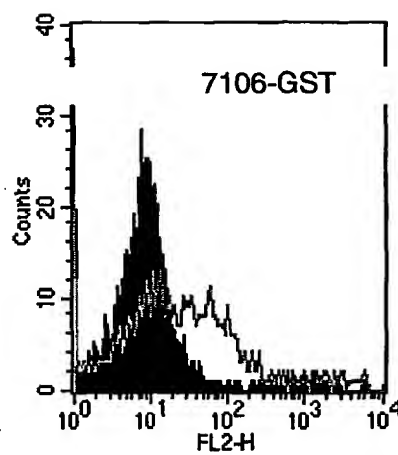
Fig. 30C



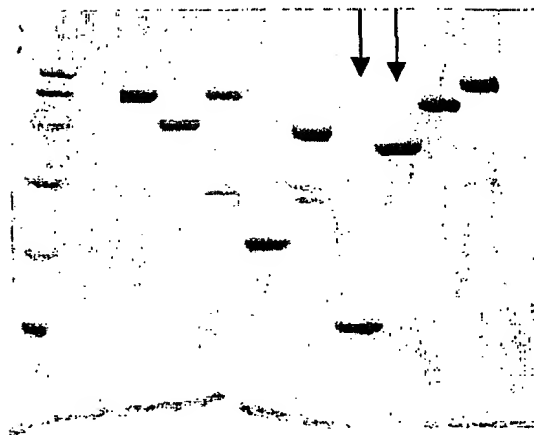
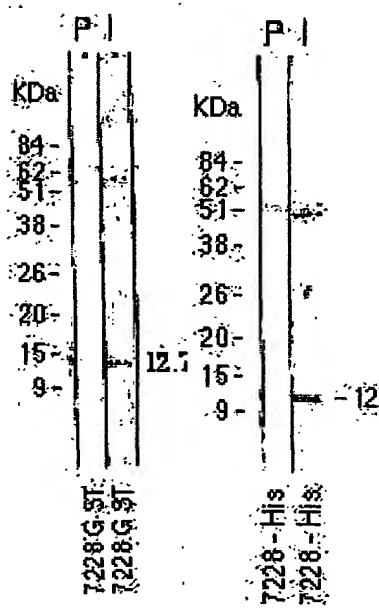
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FIGURE 31**Fig. 31A****Fig. 31B**

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FIGURE 32**Fig. 32A****Fig. 32B****Fig. 32C**

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FIGURE 33**FIG. 33A****FIG. 33B**

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FIGURE 34

Fig. 34A

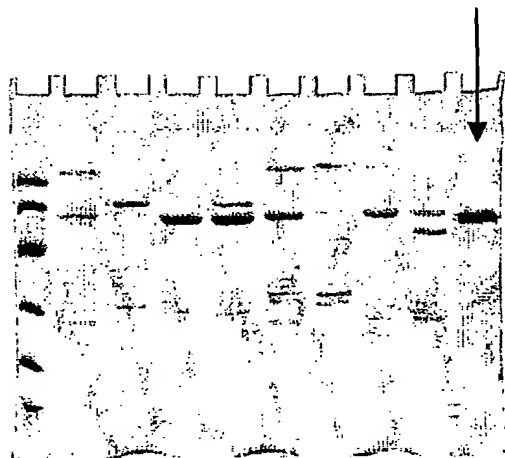
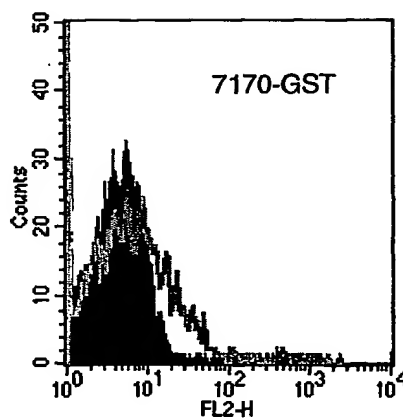


Fig. 34B



Fig. 34C



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FIGURE 35

Fig. 35A

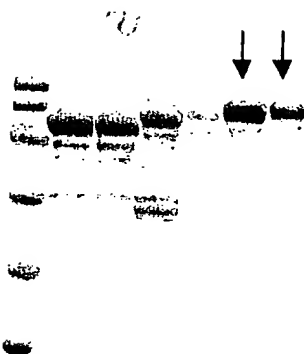


Fig. 35B

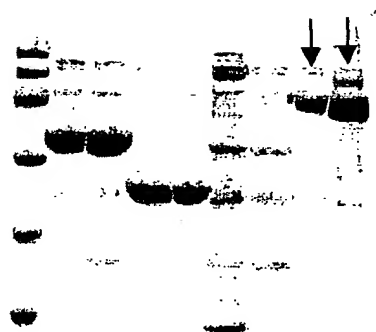
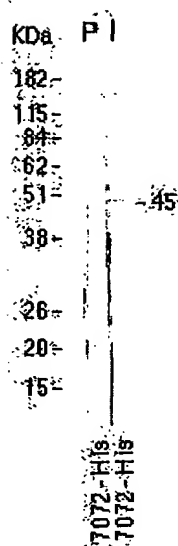
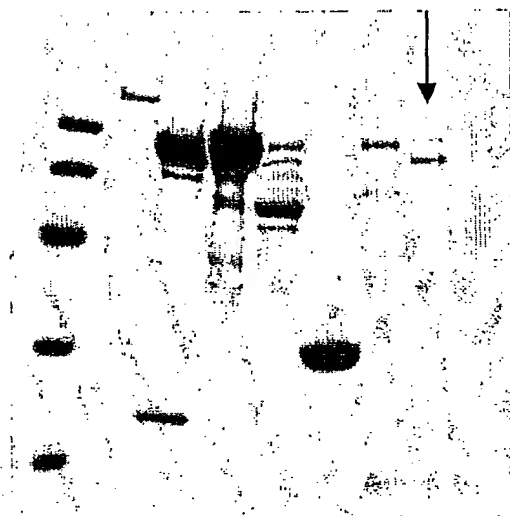
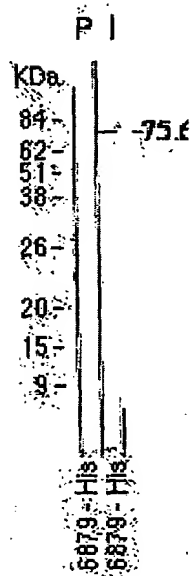


Fig. 35C



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FIGURE 36**Fig. 36A****Fig. 36B**

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FIGURE 37

FIG. 37A



FIG. 37C

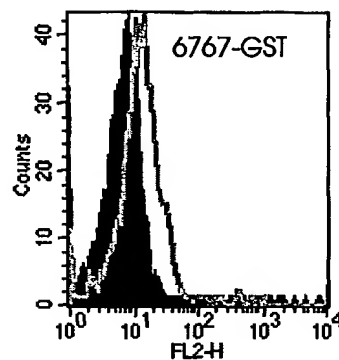


Fig. 37B

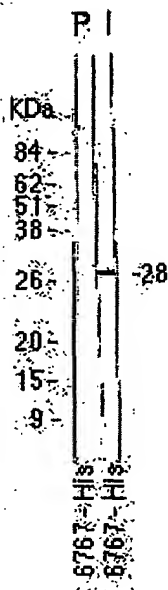
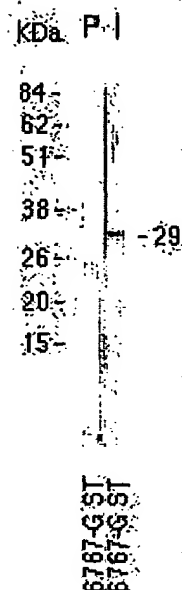
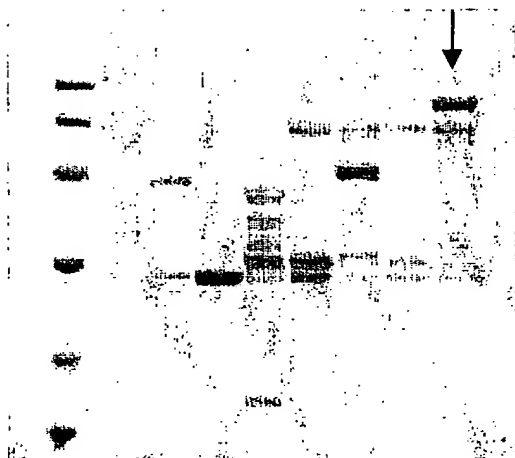
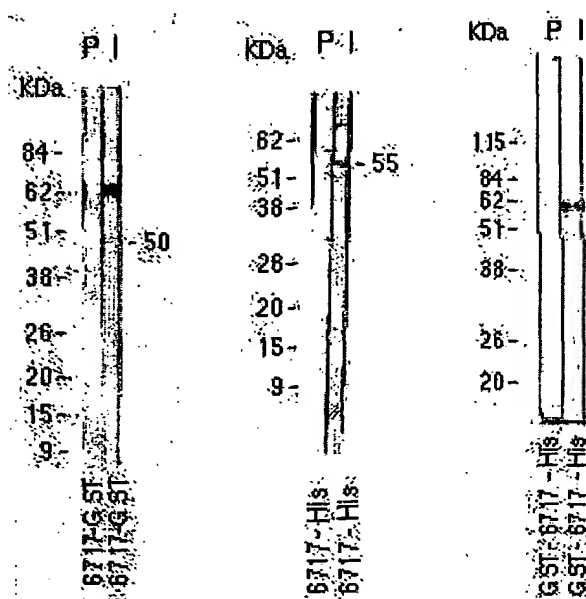


Fig. 37D



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FIGURE 38**FIG. 38A****FIG. 38B**

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FIGURE 39

Fig. 39A

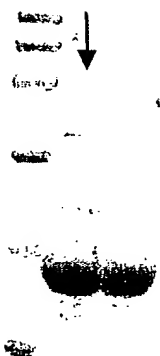


Fig. 39B

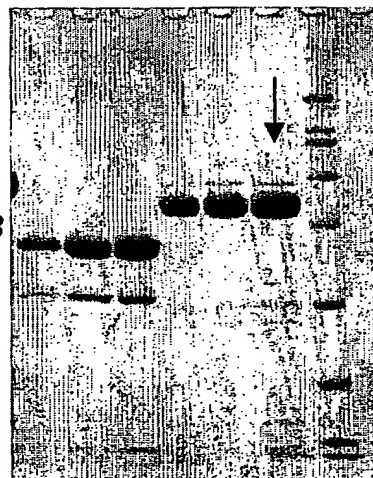


Fig. 39C

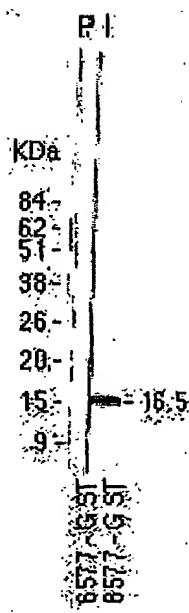
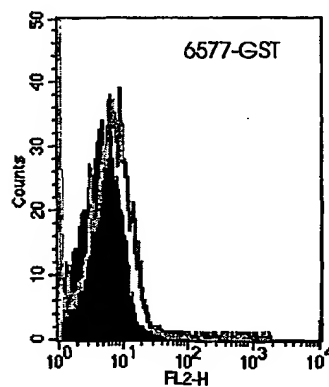


Fig. 39D



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FIGURE 40

FIG. 40A

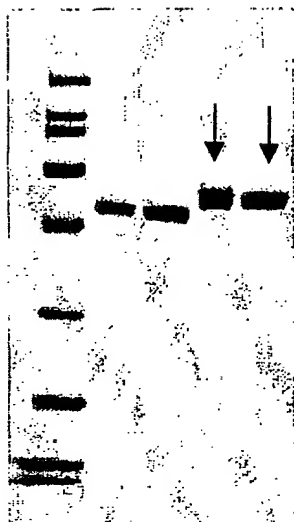


FIG. 40B



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FIGURE 41

FIG. 41A

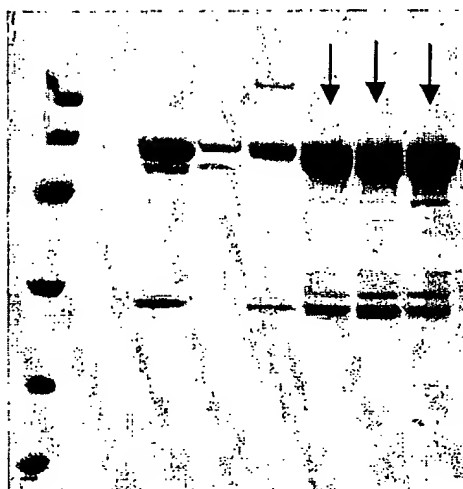


FIG. 41B

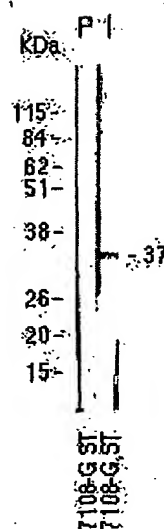
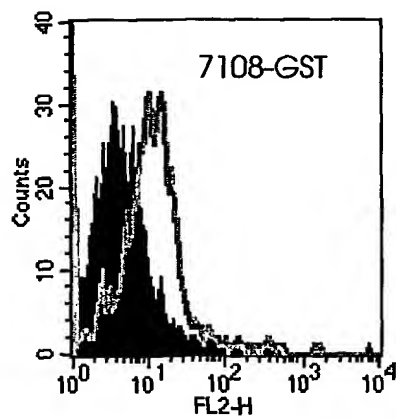


FIG. 41C



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FIGURE 42

FIG. 42A

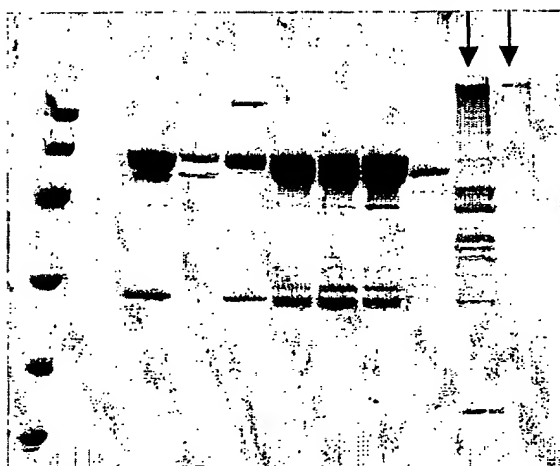


FIG. 42B

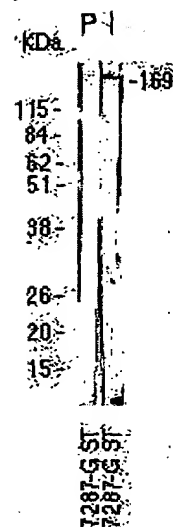
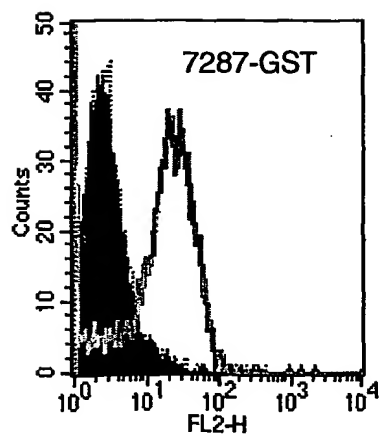


FIG. 42C



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FIGURE 43

FIG. 43A

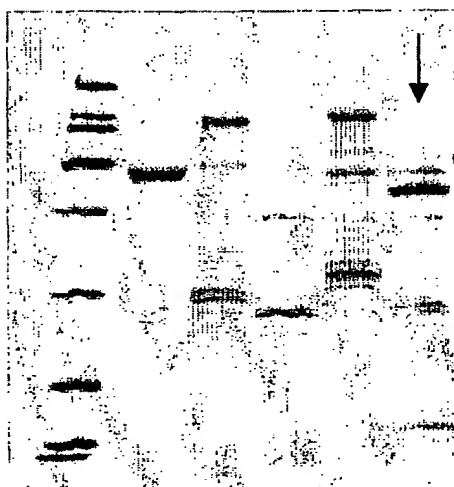


FIG. 43B

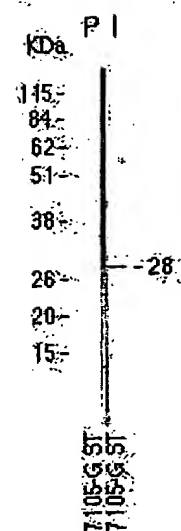
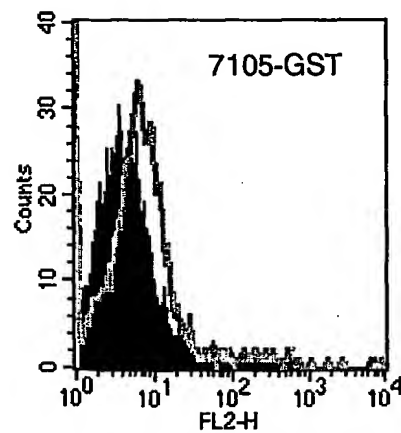


FIG. 43C



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FIGURE 44

FIG. 44A

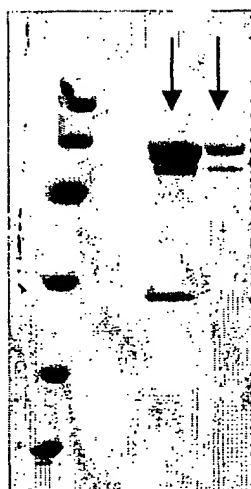


FIG. 44B

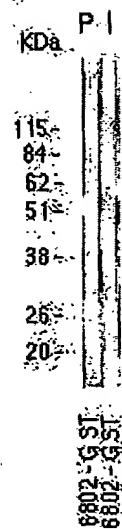
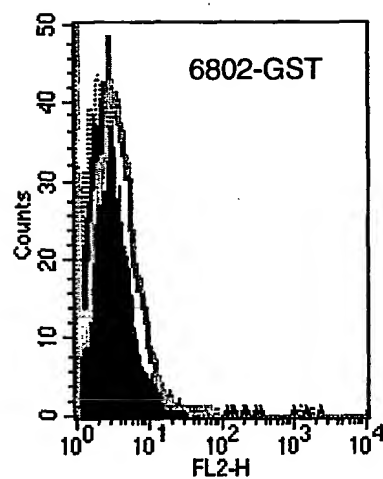


FIG. 44C



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FIGURE 45

Fig. 45A

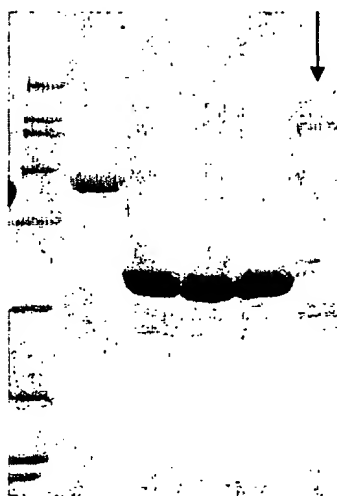


Fig. 45B

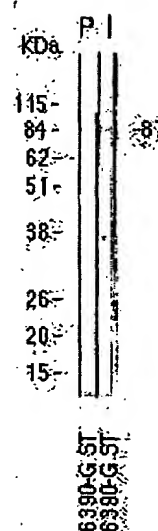
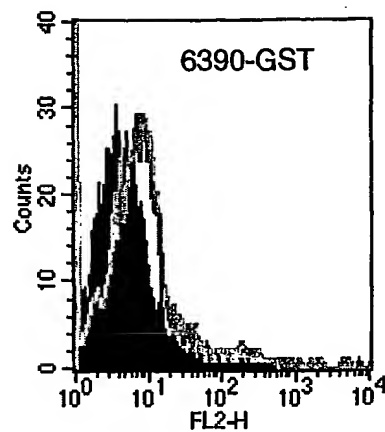


Fig. 45C



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FIGURE 46

Fig. 46A

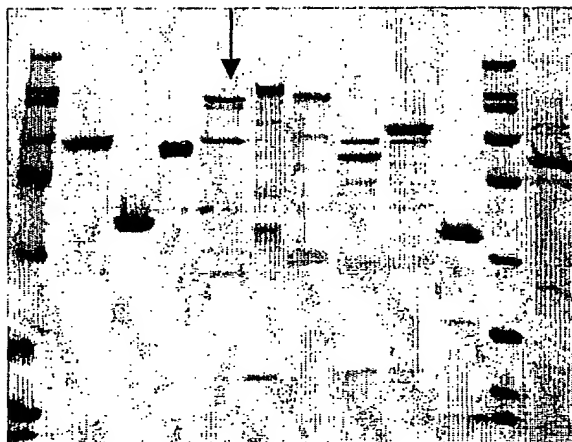
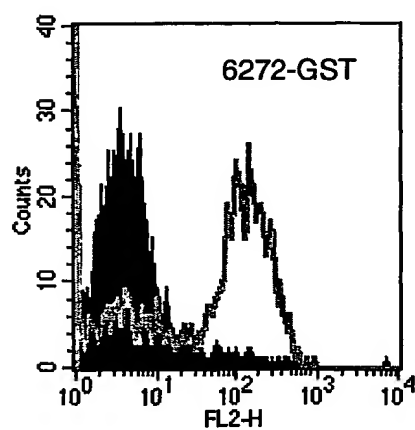


Fig. 46B



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FIGURE 47

Fig. 47A



FIG. 47B

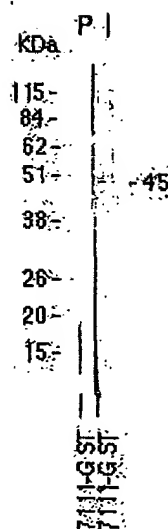
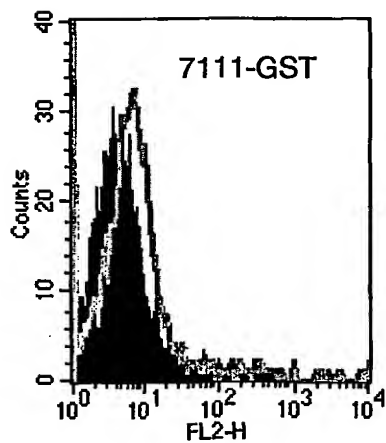


Fig. 47C



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FIGURE 48

Fig. 48A



Fig. 48B

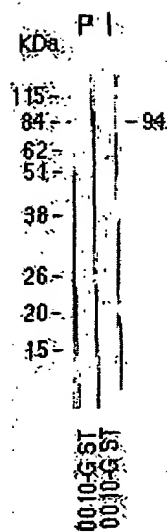
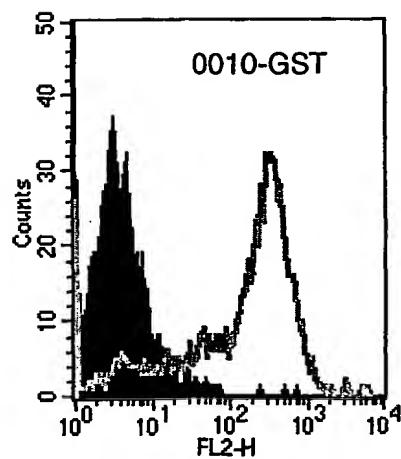


Fig. 48C



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FIGURE 49

Fig. 49A

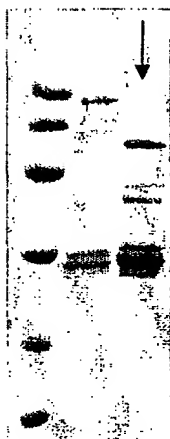


Fig. 49B

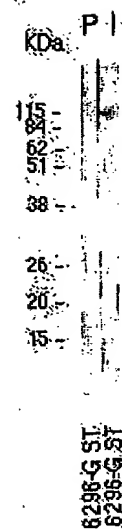
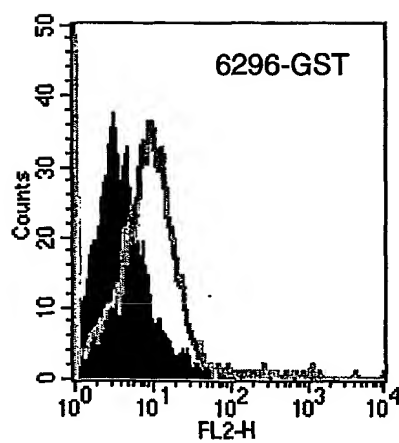


Fig. 49C



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FIGURE 50

Fig. 50A

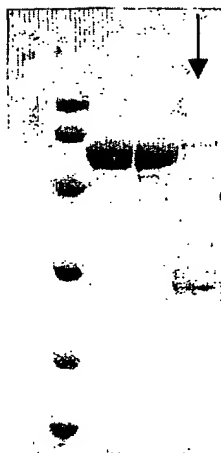


Fig. 50B

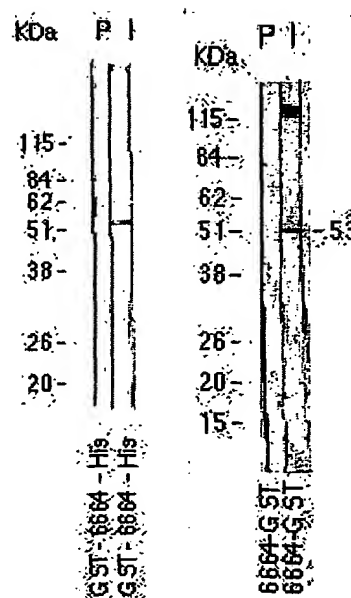
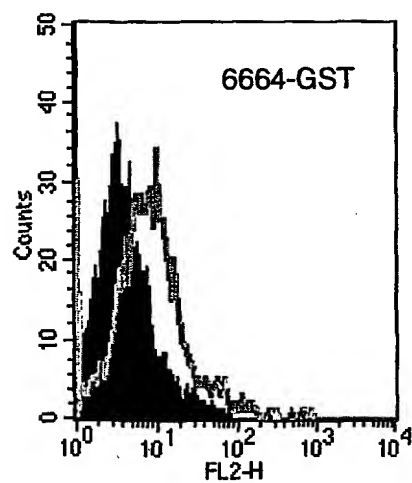


Fig. 50C



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FIGURE 51

Fig. 51A

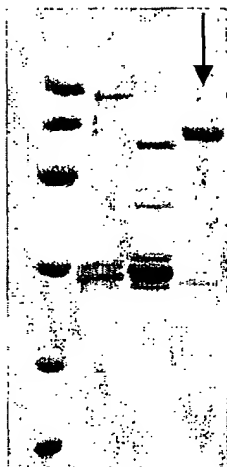


Fig. 51B

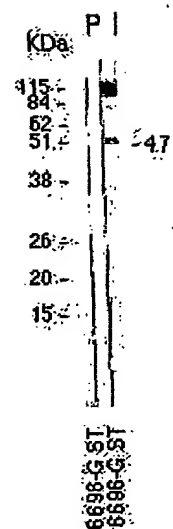
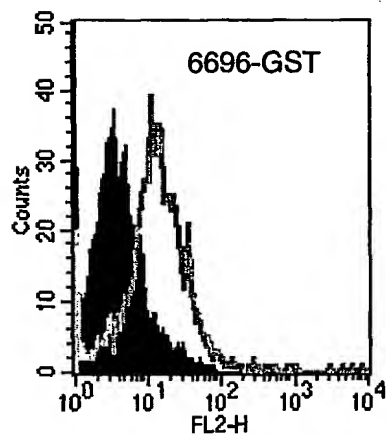


Fig. 51C



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FIGURE 52

Fig. 52A

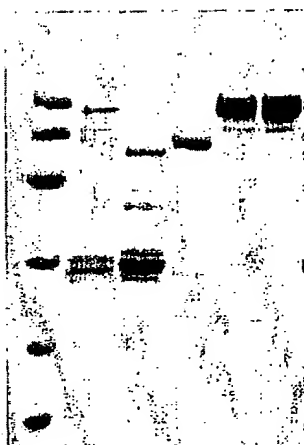


Fig. 52B

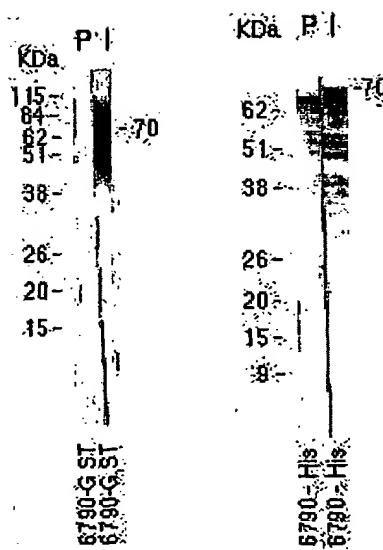
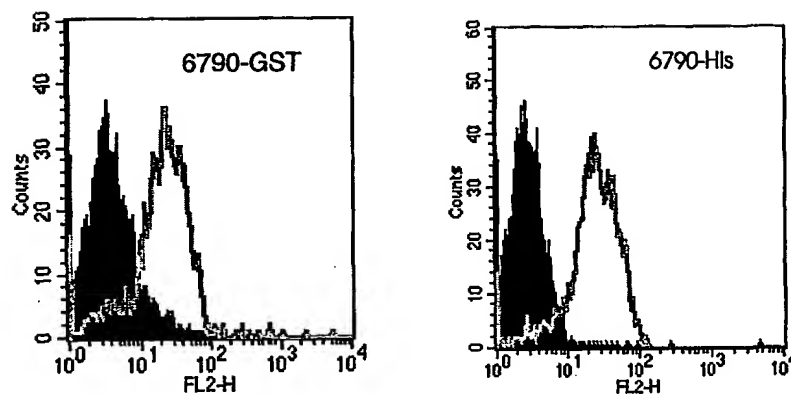
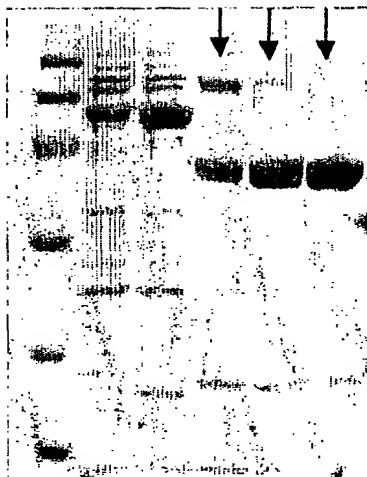
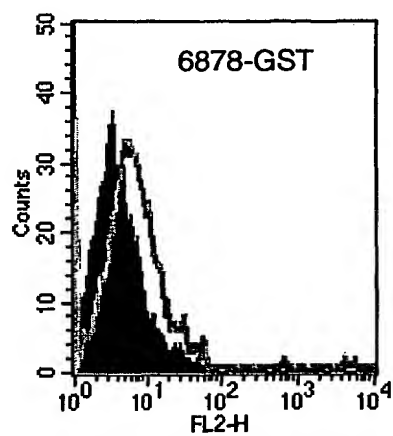


Fig. 52C



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FIGURE 53**Fig. 53A****Fig. 53B**

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FIGURE 54

Fig. 54A

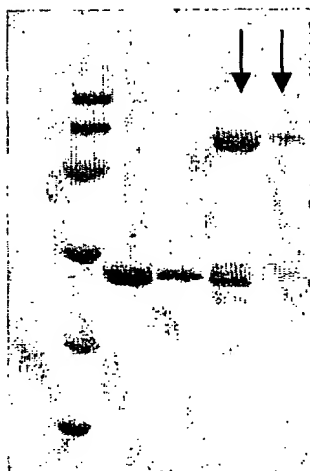


Fig. 54B

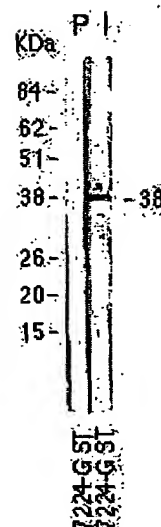
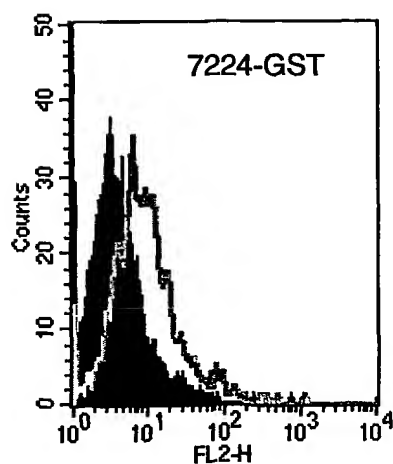


Fig. 54C



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FIGURE 55

Fig. 55A

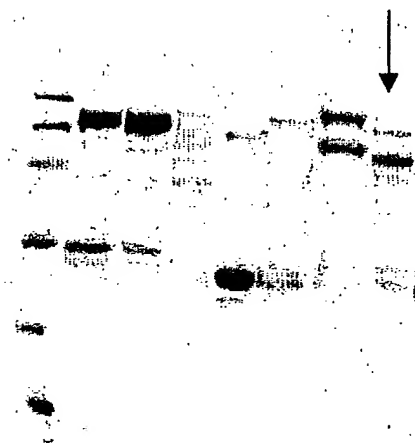


Fig. 55B

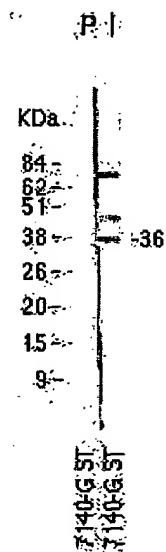
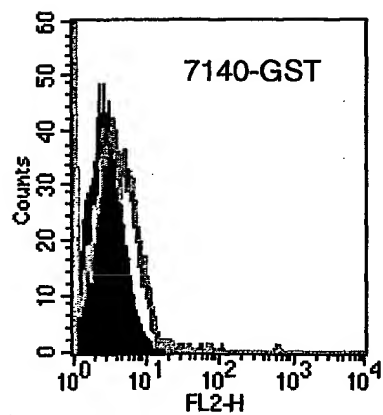


Fig. 55C



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FIGURE 56

FIG. 56A



FIG. 56B

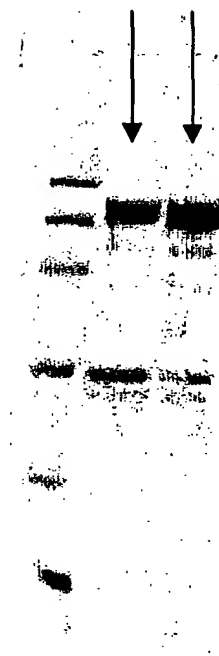


FIG. 56C

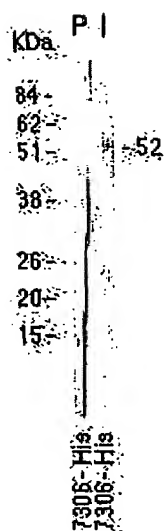
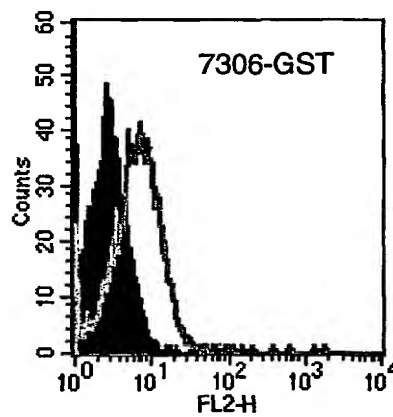


FIG. 56D



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FIGURE 57

Fig. 57A

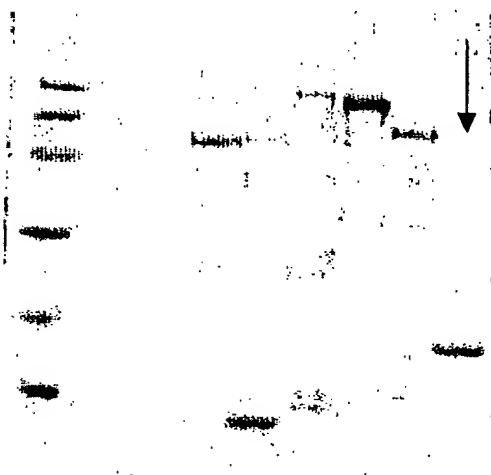


Fig. 57B

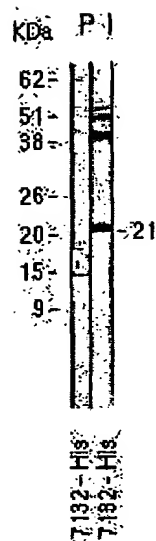
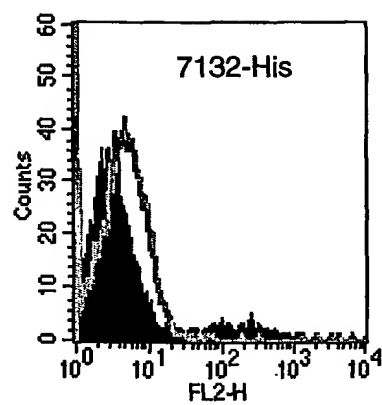


Fig. 57C



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FIGURE 58

FIG. 58A

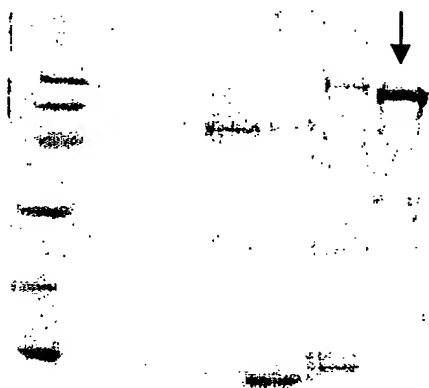


FIG. 58B

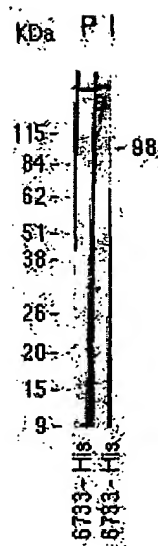
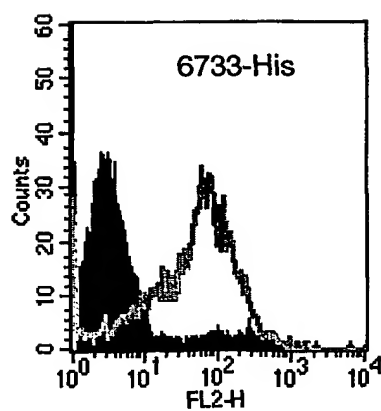


FIG. 58C



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FIGURE 59

Fig. 59A

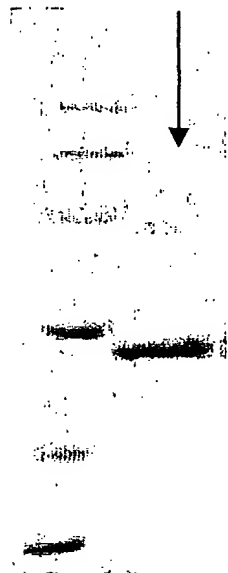


Fig. 59B

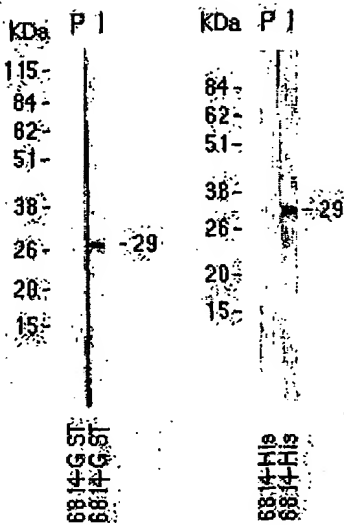
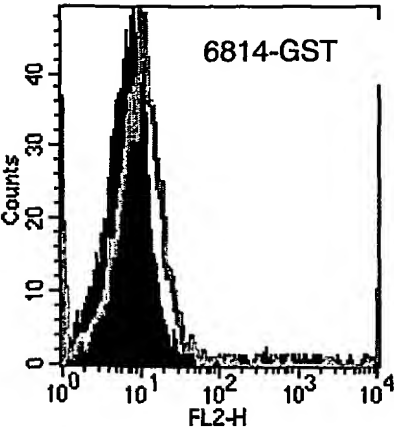


Fig. 59C



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FIGURE 60

FIG. 60A

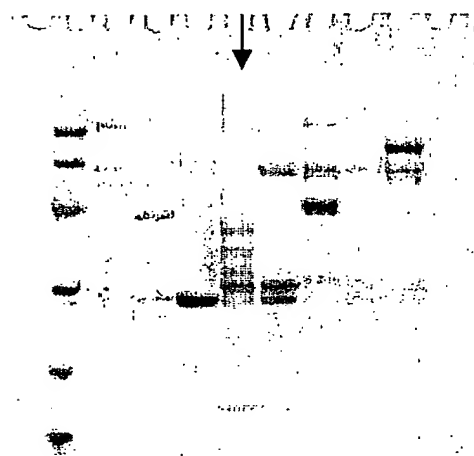
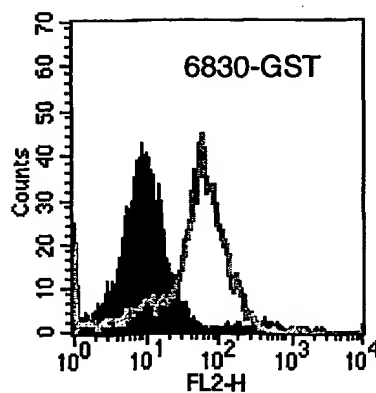


FIG. 60B



FIG. 60C



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FIGURE 61

FIG. 61A

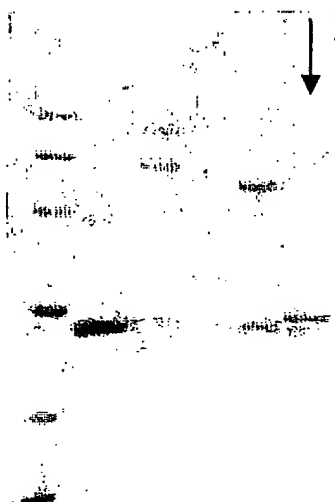


FIG. 61B

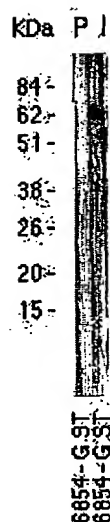
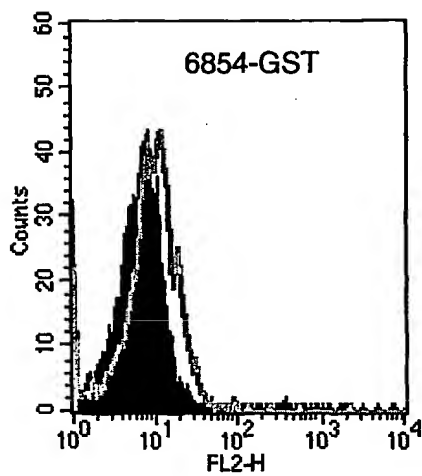
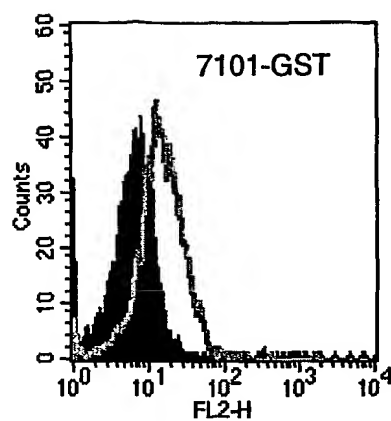
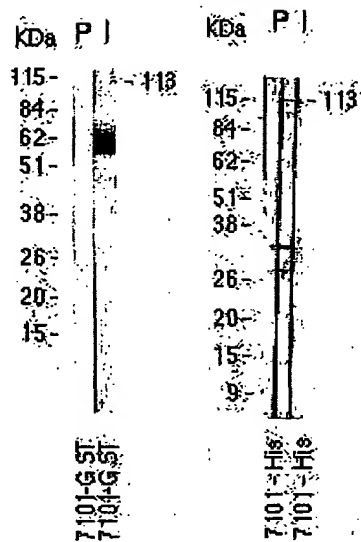


FIG. 61C



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FIGURE 62**Fig. 62A****Fig. 62C****Fig. 62B**

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FIGURE 63

FIG. 63A

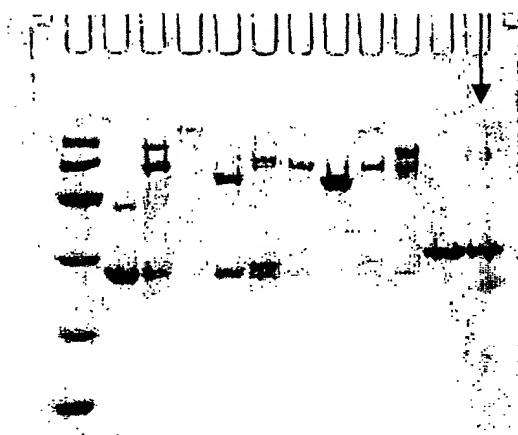


FIG. 63B

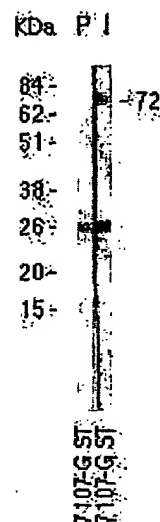
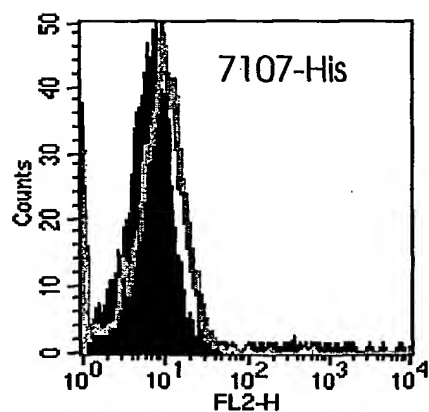


FIG. 63C



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FIGURE 64

FIG. 64A

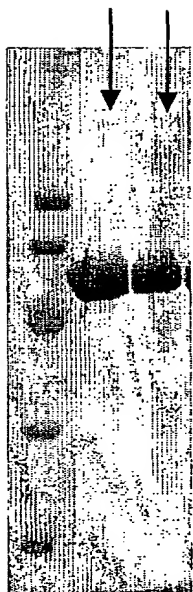


FIG. 64B

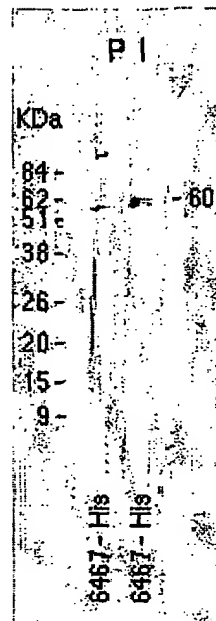


FIG. 64C

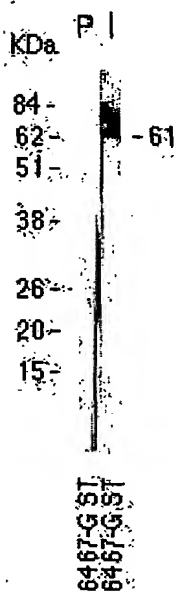
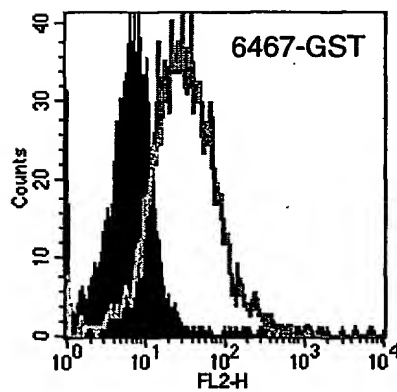


FIG. 64D



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FIGURE 65

Fig. 65A

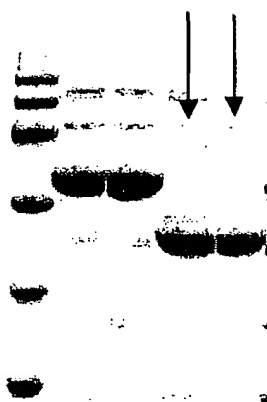


Fig. 65B

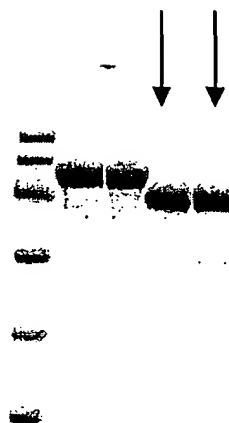
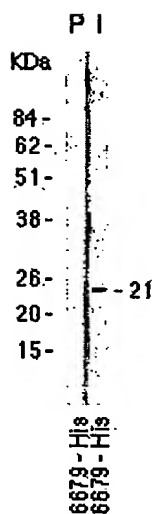


Fig. 65C



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FIGURE 66



Fig. 66A

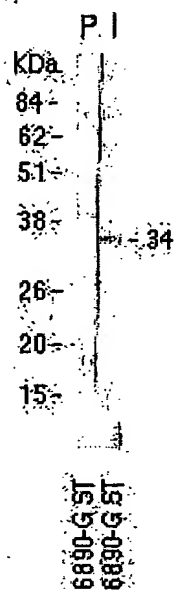


Fig. 66B

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FIGURE 68

FIG. 68A

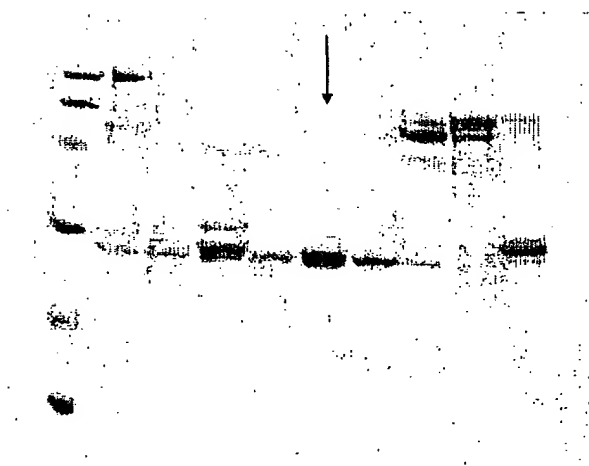
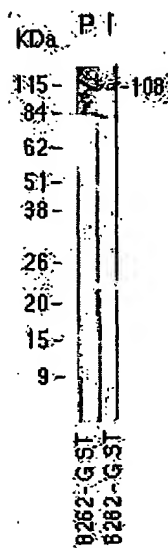


FIG. 68B



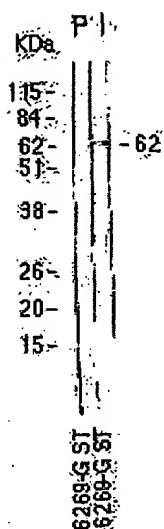
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FIGURE 69

Fig. 69A



Fig. 69B



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FIGURE 70

Fig. 70A

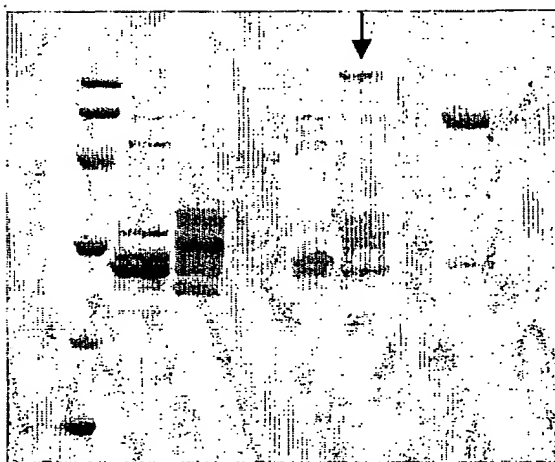
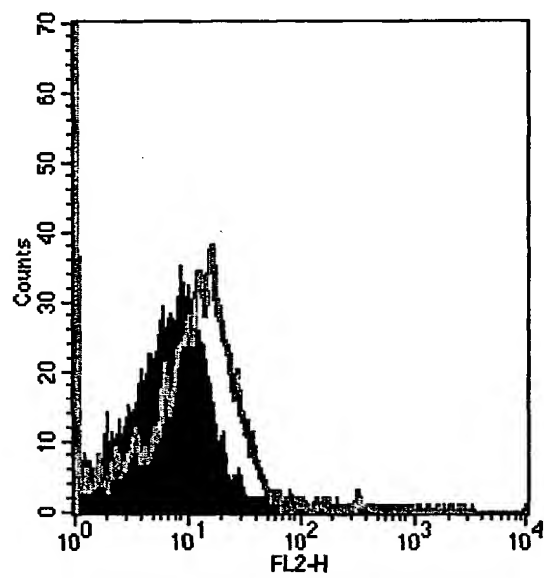


Fig. 70B



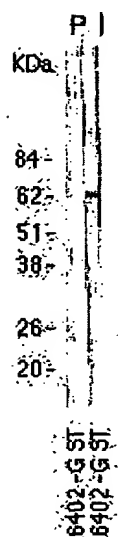
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FIGURE 71

FIG. 71A



FIG. 71B



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FIGURE 72

FIG. 72A

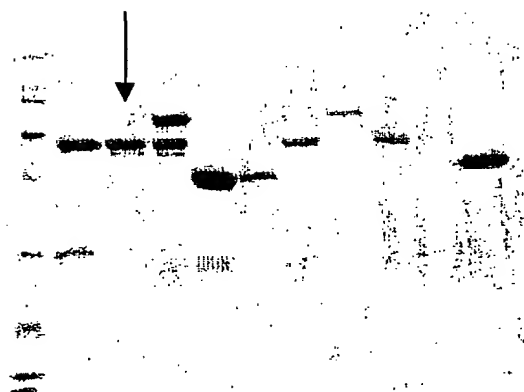
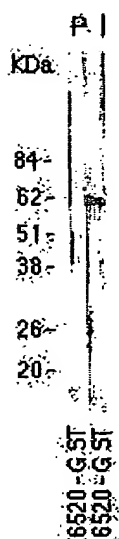


FIG. 72B



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FIGURE 73

Fig. 73A

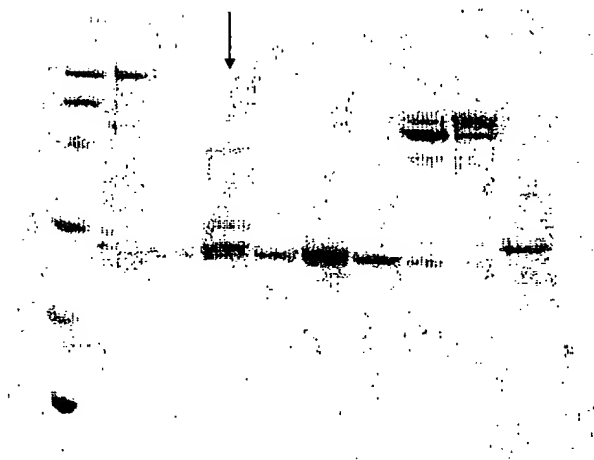
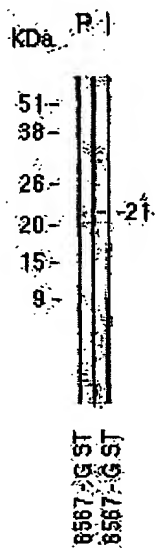


Fig. 73B



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FIGURE 74

FIG. 74A



FIG. 74B

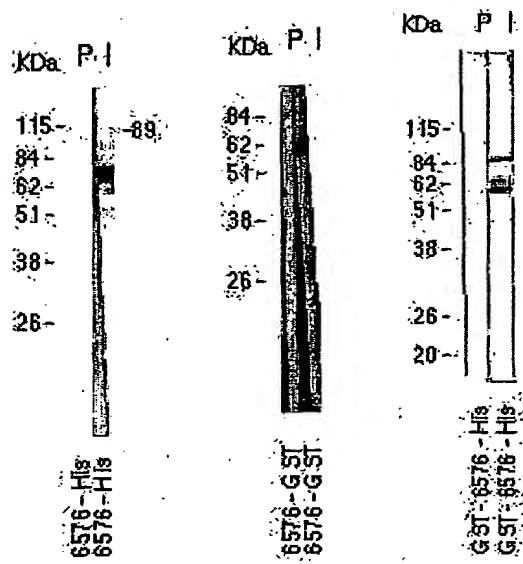
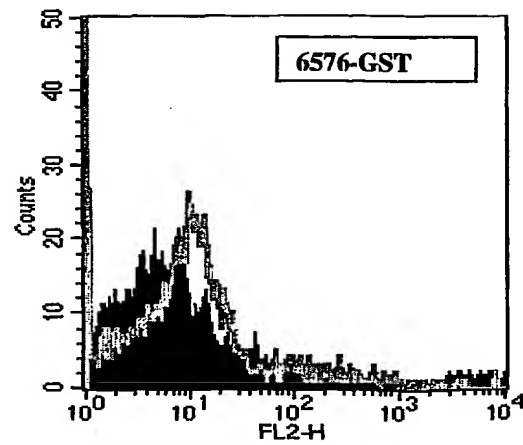


FIG. 74C



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FIGURE 75

Fig. 75A

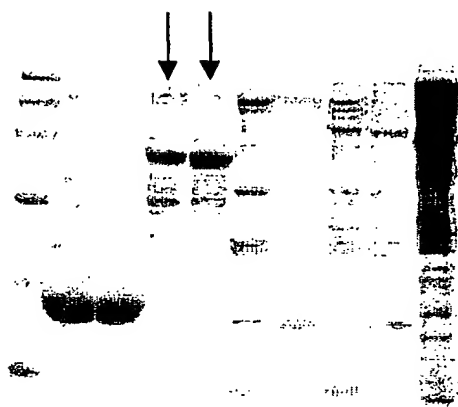
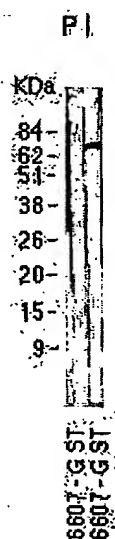


Fig. 75B



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FIGURE 76

FIG. 76A

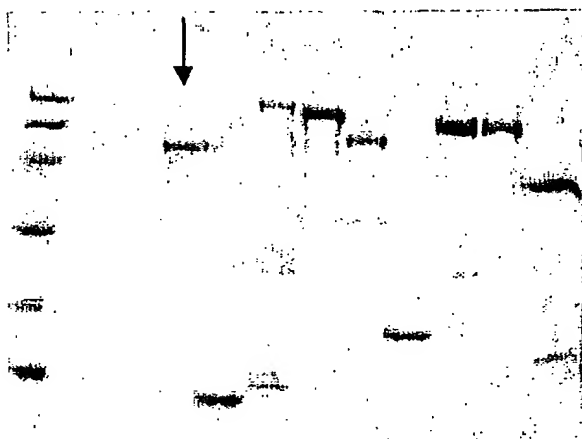
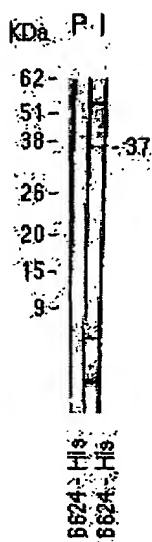


FIG. 76B



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FIGURE 77

Fig. 77A

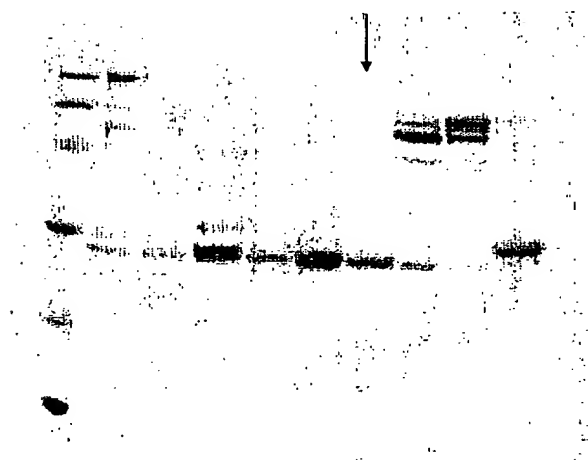
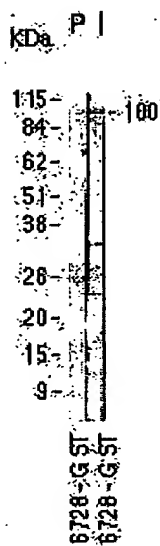


Fig. 77B



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FIGURE 78

Fig. 78A

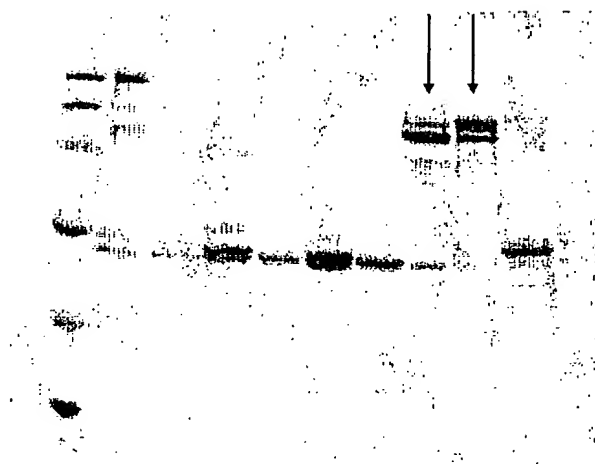
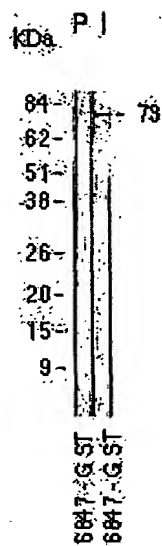


Fig. 78B



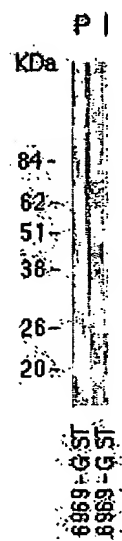
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FIGURE 79

Fig. 79A



Fig. 79B



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FIGURE 81

Fig. 81A

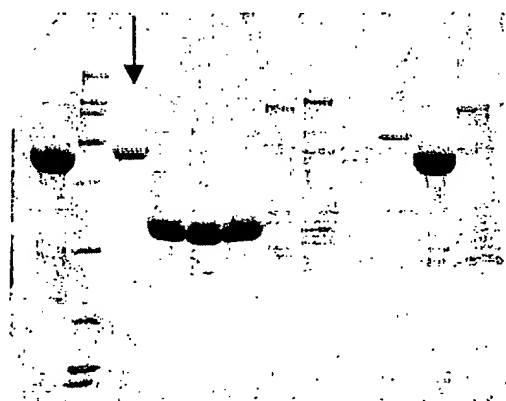
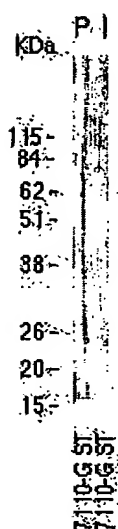


Fig. 81B



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FIGURE 82

Fig. 82A

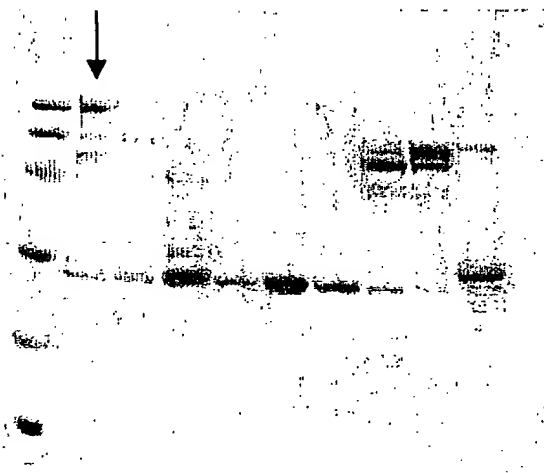
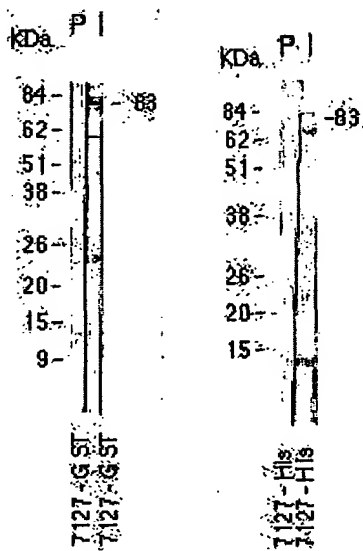
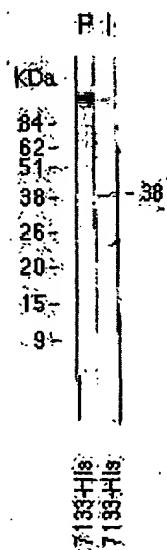
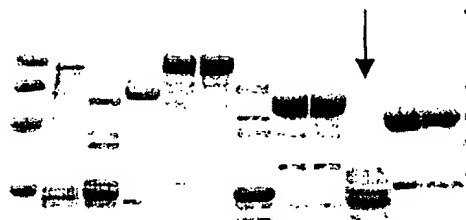


Fig. 82B



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FIGURE 83



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FIGURE 84

Fig. 84A

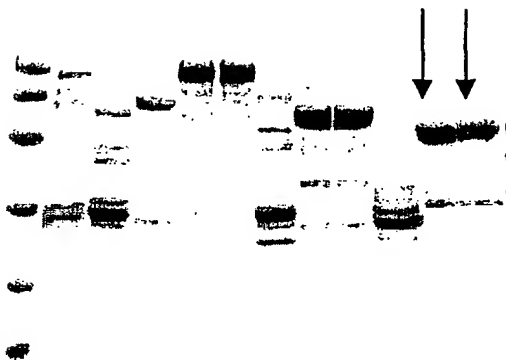
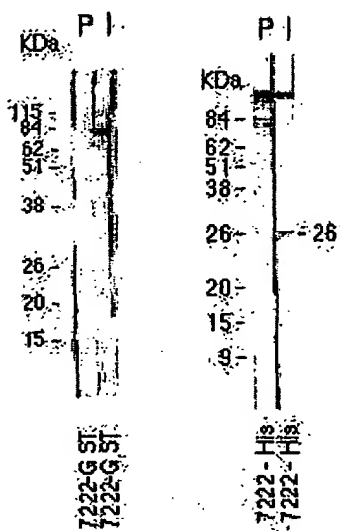


Fig. 84B



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FIGURE 85

Fig. 85A

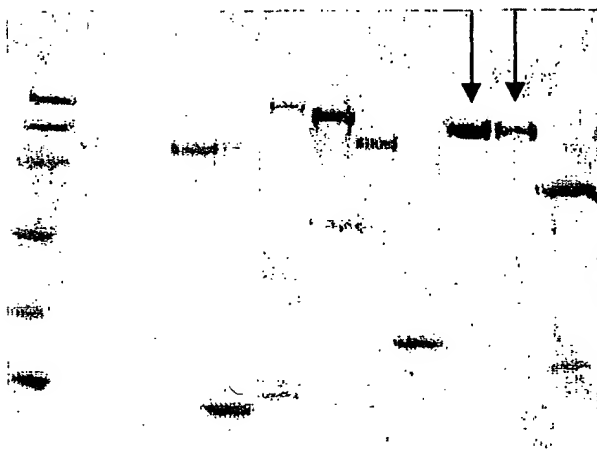
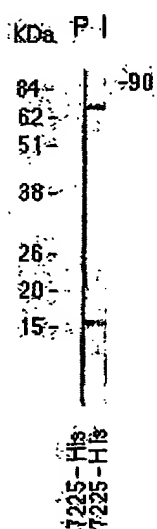


Fig. 85B



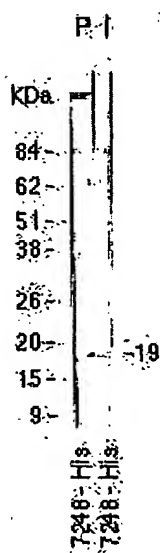
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FIGURE 86

Fig. 86A



Fig. 86B



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FIGURE 87

Fig. 87A

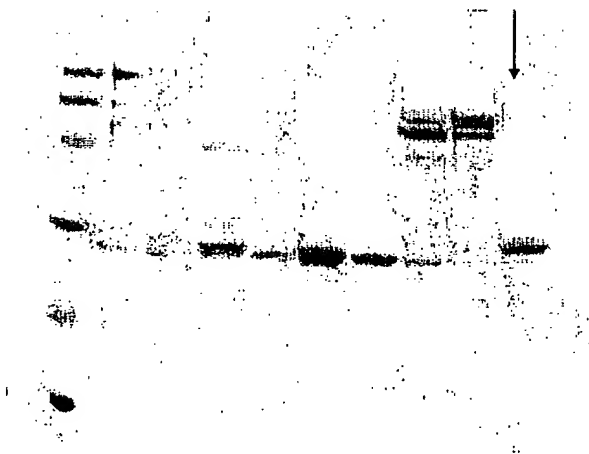
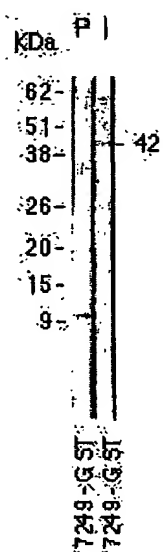


Fig. 87B



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FIGURE 88

Fig. 88A

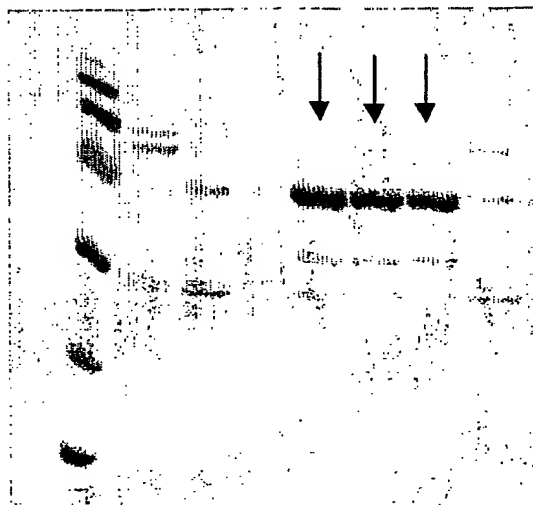
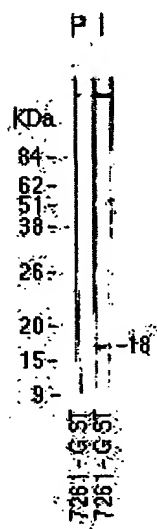


Fig. 88B



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FIGURE 89

Fig. 89A

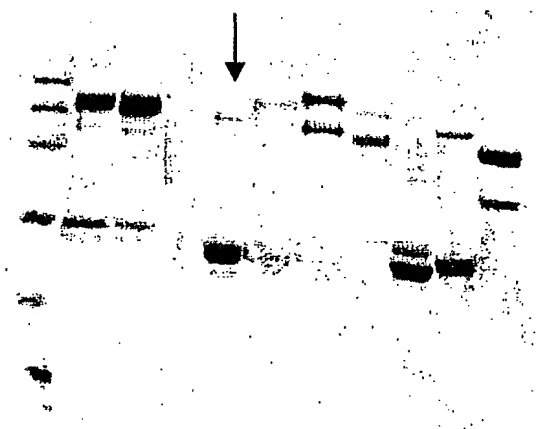
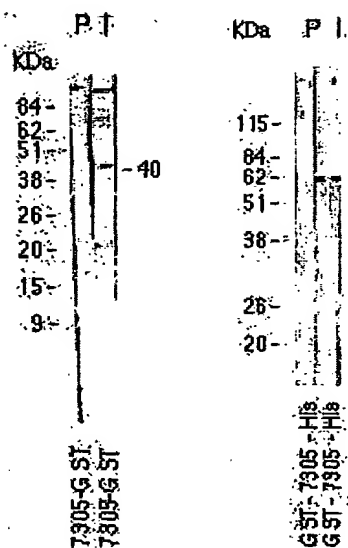


Fig. 89B



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FIGURE 90

Fig. 90A

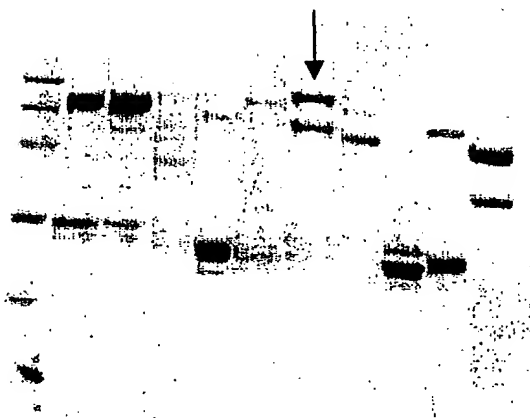
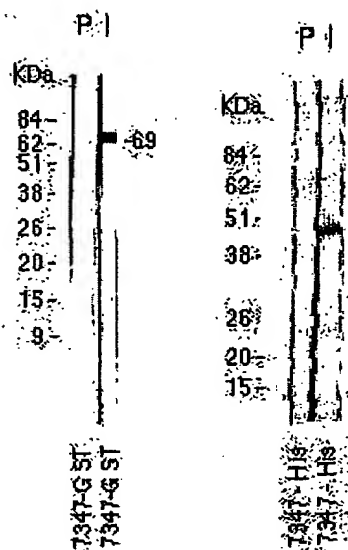


Fig. 90B



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FIGURE 91

Fig. 91A

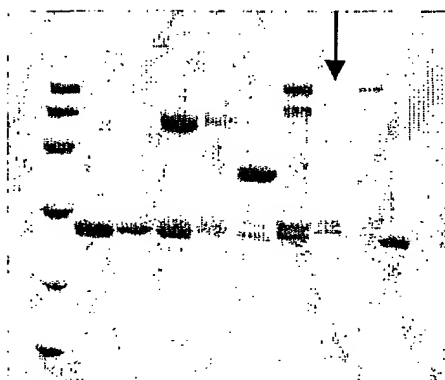
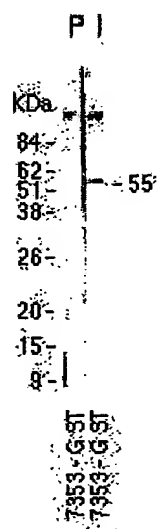


Fig. 91B



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FIGURE 92

FIG. 92A

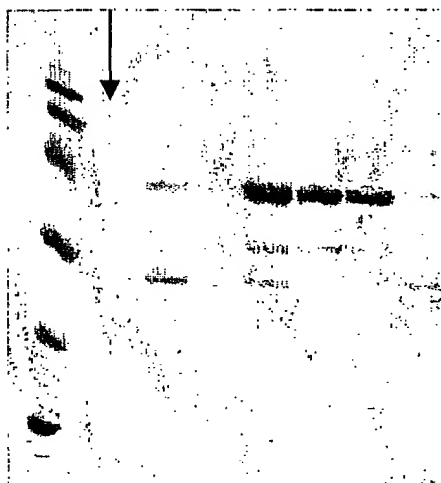
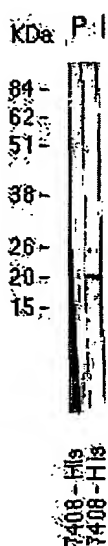


FIG. 92B



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FIGURE 93

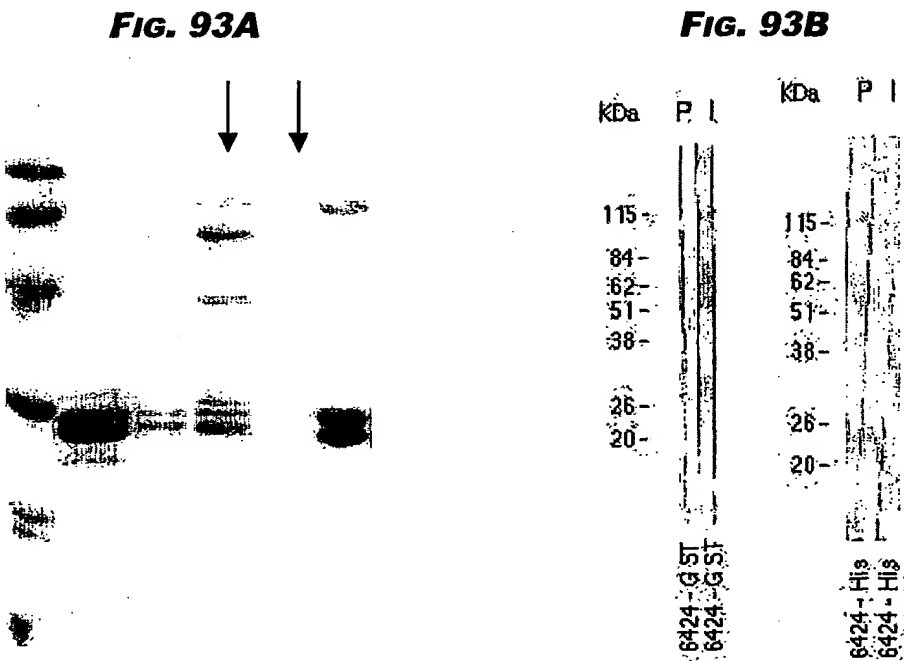
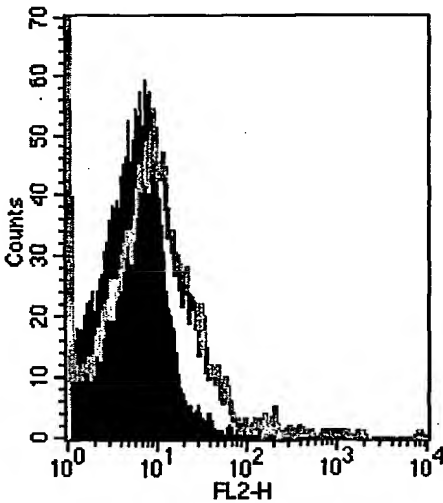


FIG. 93C



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FIGURE 94

FIG. 94A

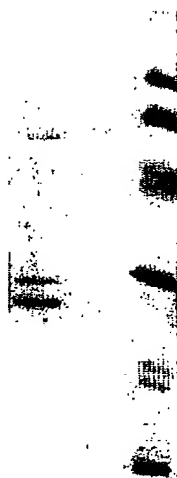


FIG. 94B

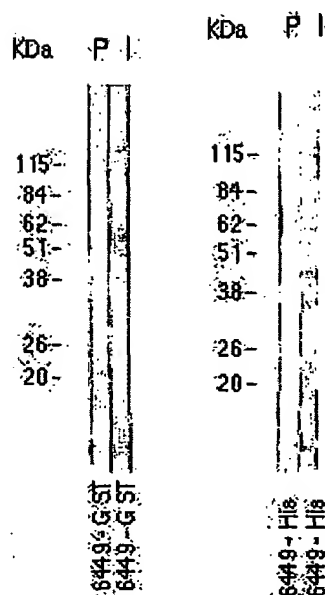
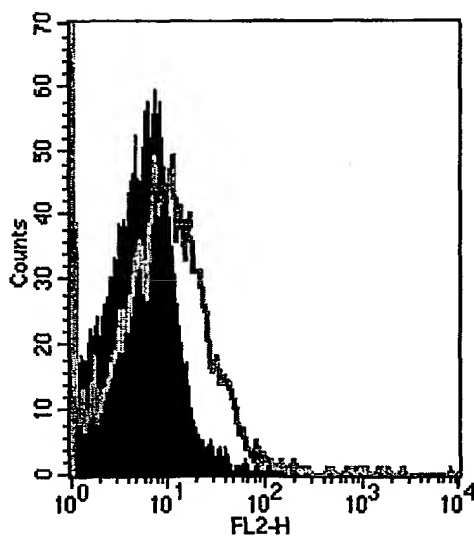


FIG. 94C



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FIGURE 95

FIG. 95A



FIG. 95B

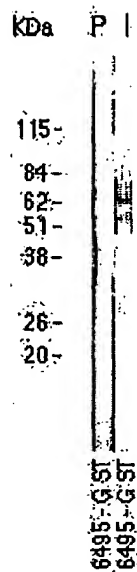
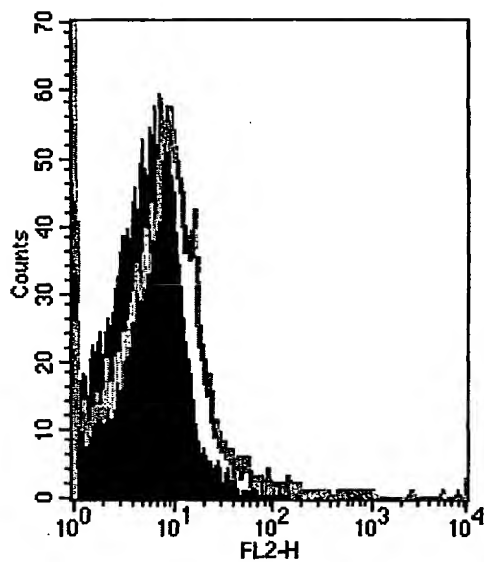


FIG. 95C



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FIGURE 96

FIG. 96A

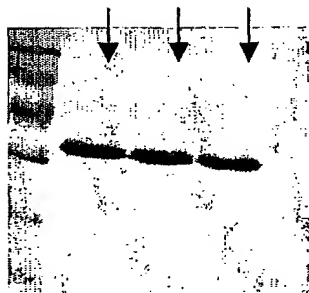


FIG. 96B



FIG. 96C

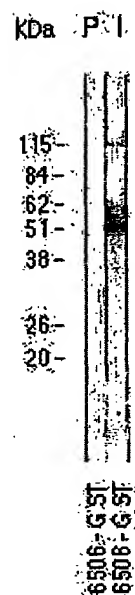
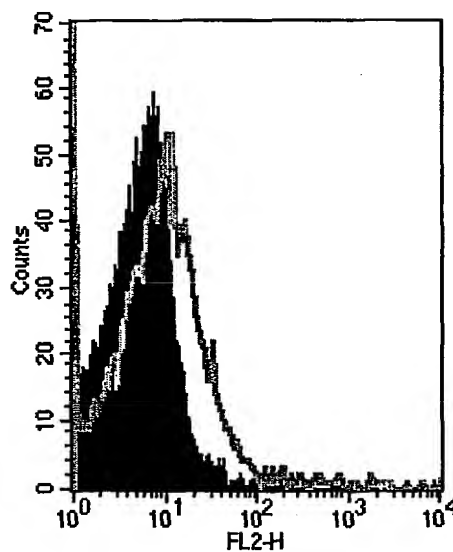


FIG. 96D



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FIGURE 97

Fig. 97A



Fig. 97B

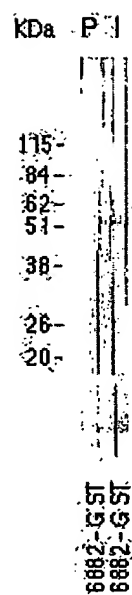
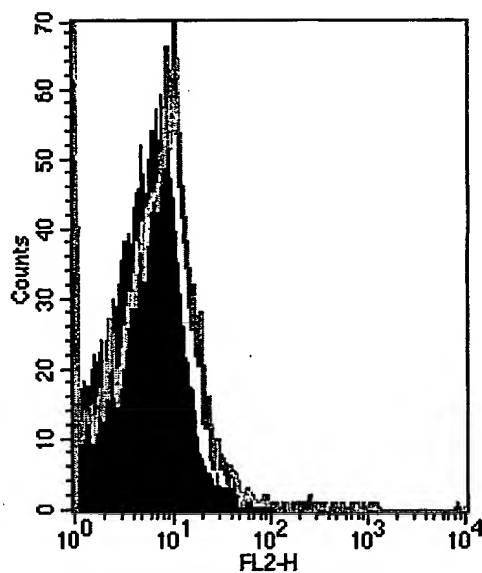


Fig. 97C



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FIGURE 98

FIG. 98A

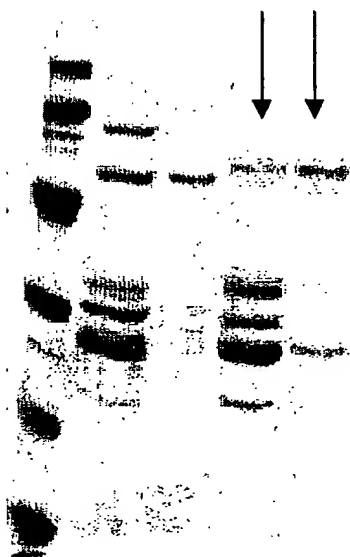


FIG. 98B

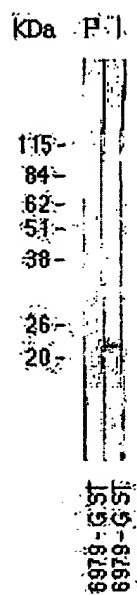
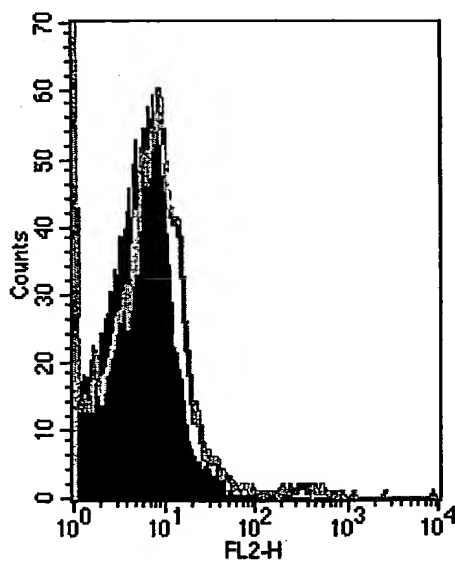


FIG. 98C



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FIGURE 99

FIG. 99A



FIG. 99B

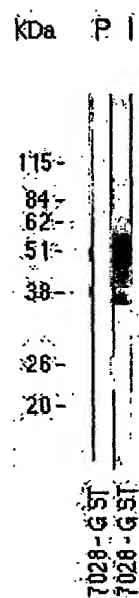
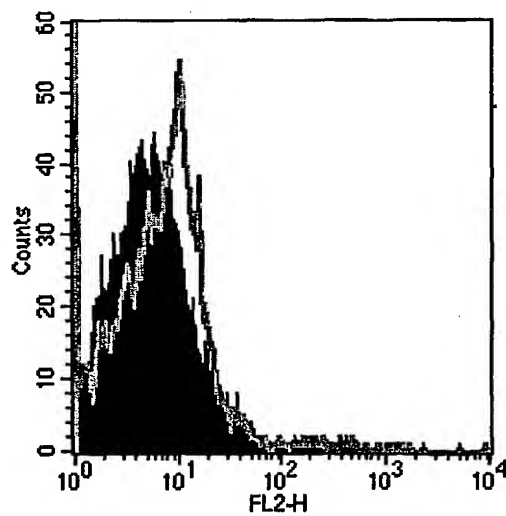


FIG. 99C



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FIGURE 100

Fig. 100A

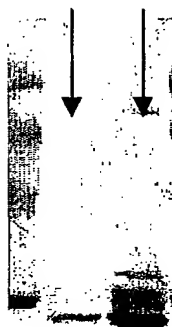


Fig. 100B

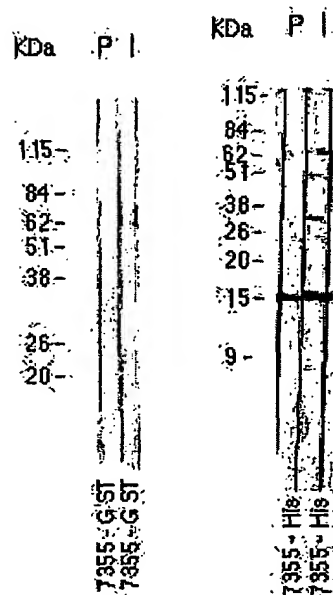
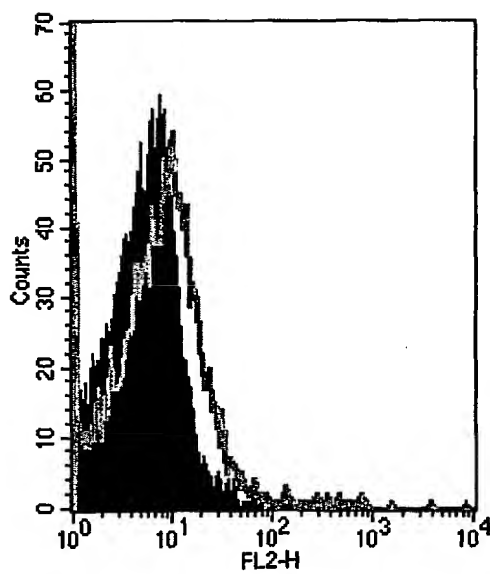


Fig. 100C



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FIGURE 101

FIG. 101A



FIG. 101B

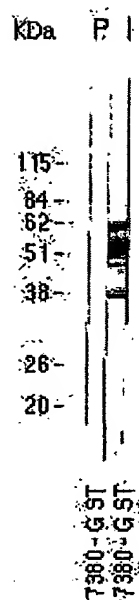
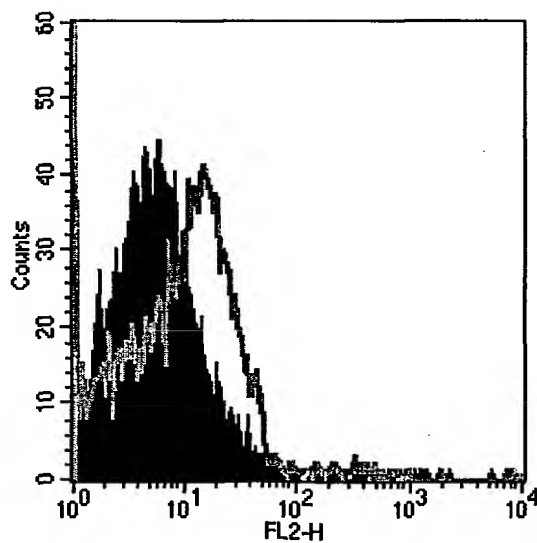


FIG. 101C



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FIGURE 102

Fig. 102A

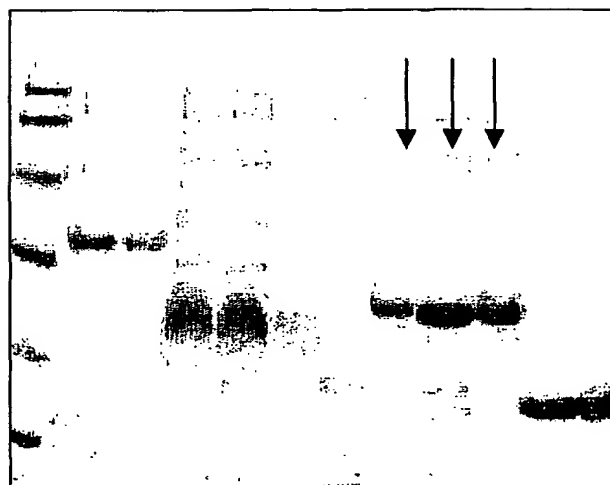
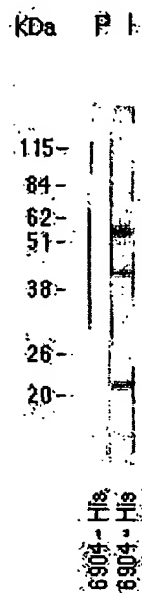


Fig. 102B



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FIGURE 103

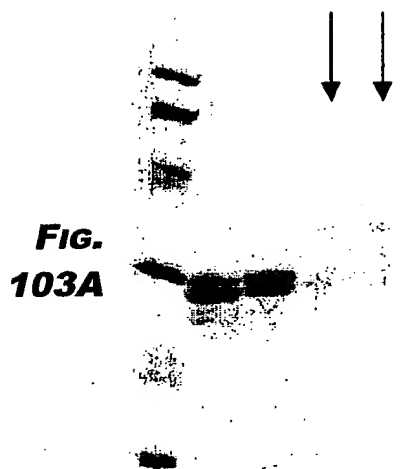


FIG. 103C

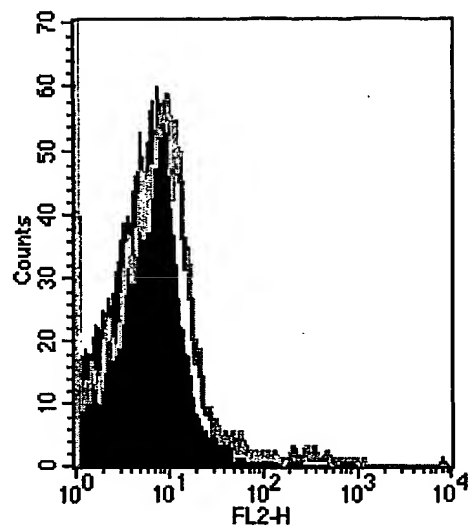
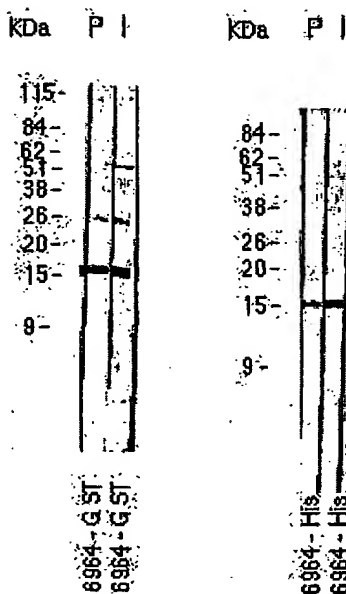


FIG. 103B



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FIGURE 104

Fig. 104A

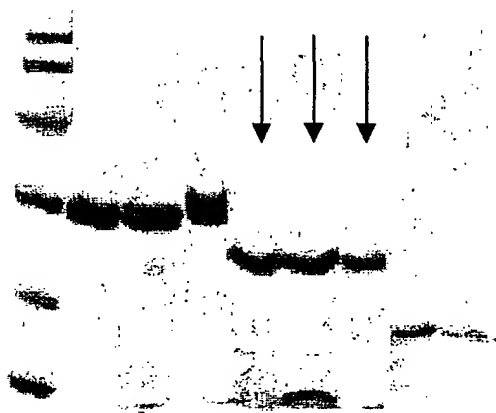


FIG. 104B

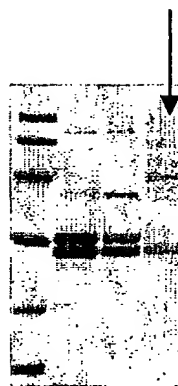
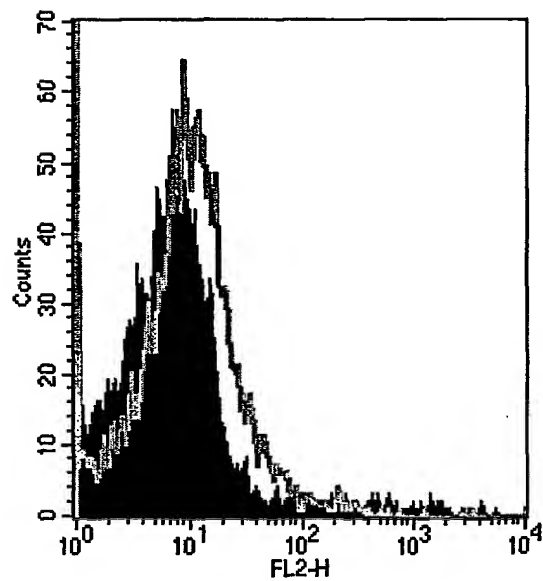


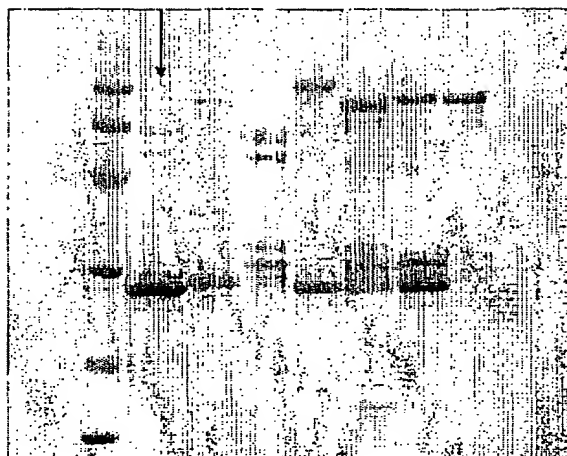
Fig. 104C



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FIGURE 105

Fig. 105A



KDa P I

Fig. 105B

115-
84-
62-
51-
38-
26-
20-

6201-G-5T
6201-G-5T

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FIGURE 106

Fig. 106A

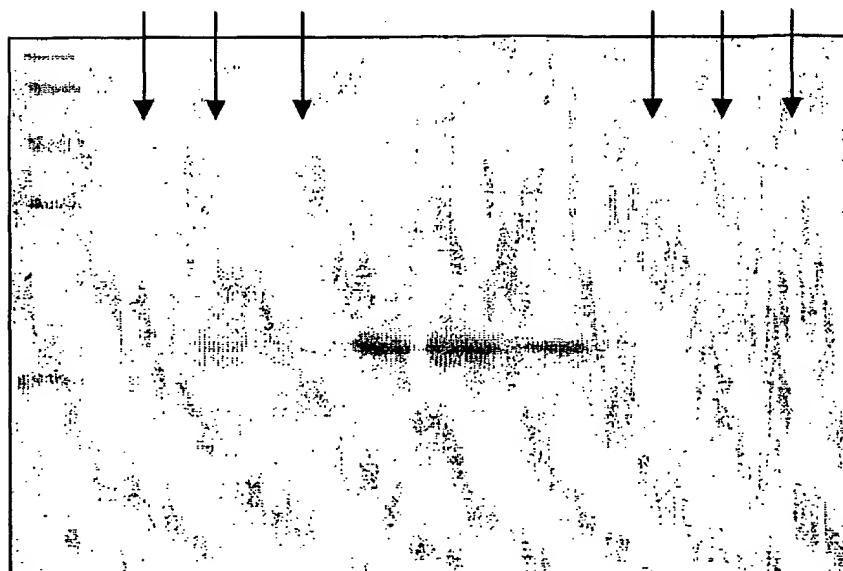
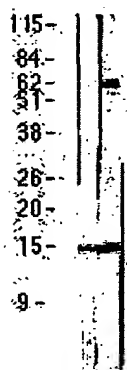


FIG. 106B

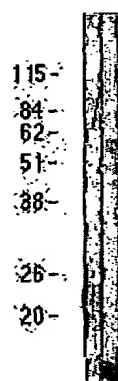
kDa P I.



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6306 6306

FIGURE 107

kDa P I.



His His
6434 6434

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FIGURE 108

Fig. 108A

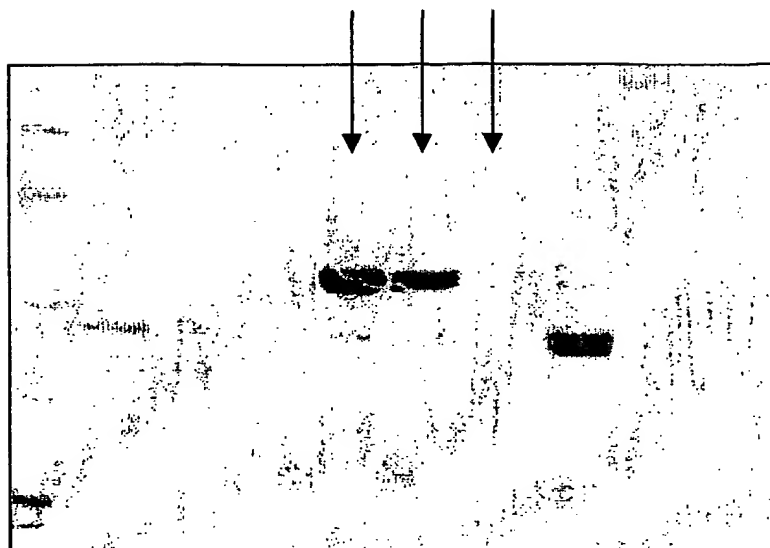
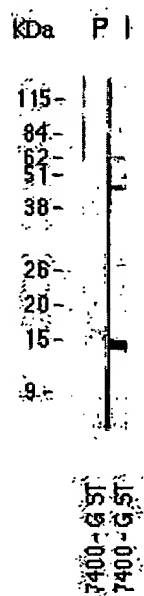


Fig. 108B



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FIGURE 109

Fig. 109A

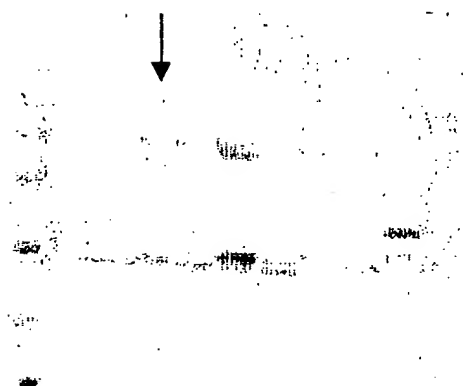
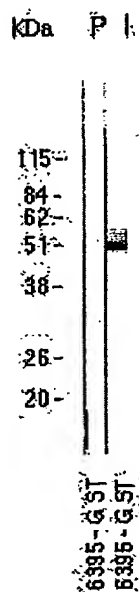


Fig. 109B



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FIGURE 110

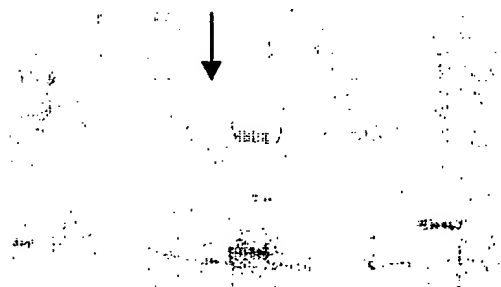


Fig. 110A



Fig. 110B

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FIGURE 111

FIG. 111A

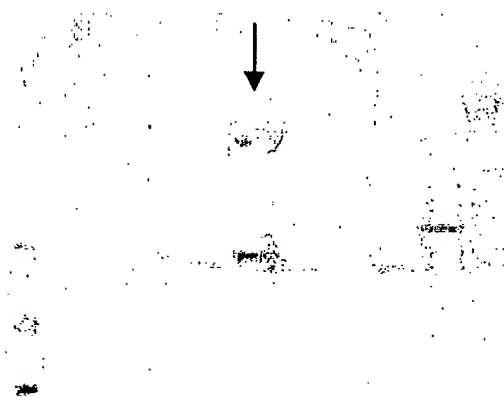
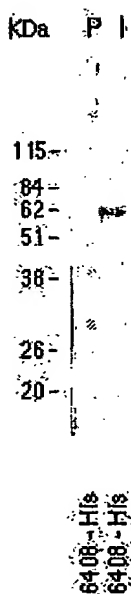


FIG. 111B



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FIGURE 112



FIG. 112A

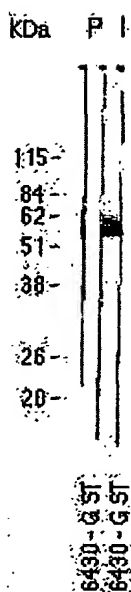


FIG. 112B

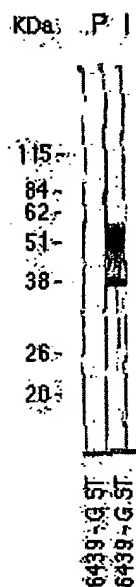
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FIGURE 113

Fig. 113A



Fig. 113B



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FIGURE 114

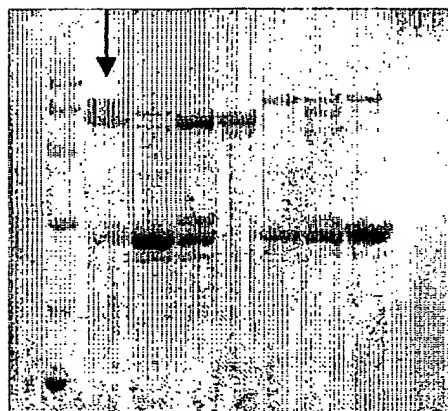


FIG. 114A

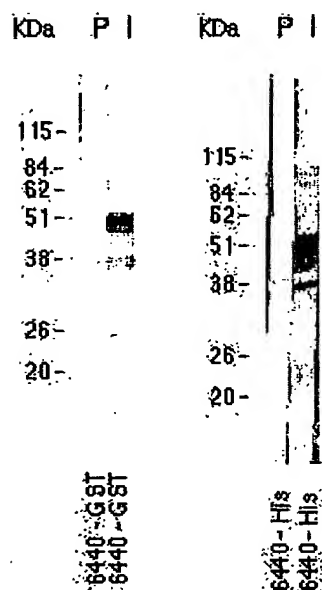


FIG. 114B

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FIGURE 115

Fig. 115A

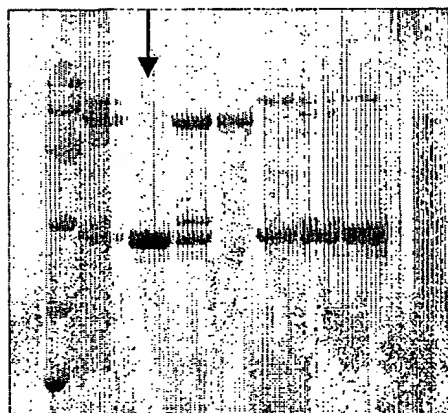
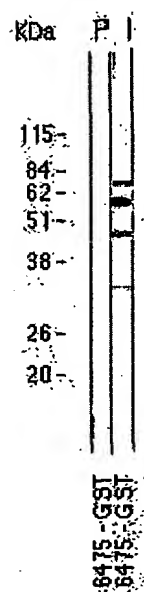


Fig. 115B



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FIGURE 116

FIG. 116A

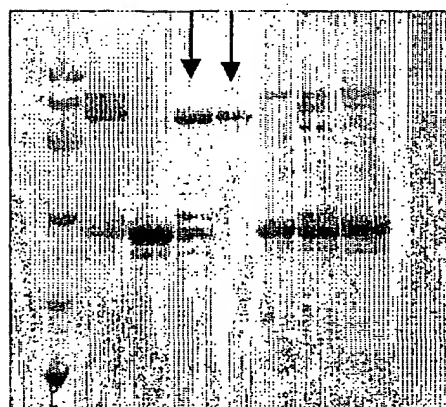
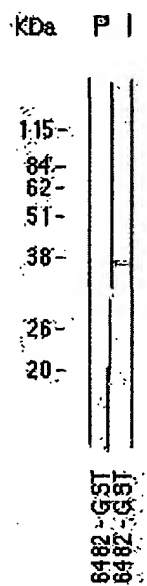


FIG. 116B



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FIGURE 117

Fig. 117A

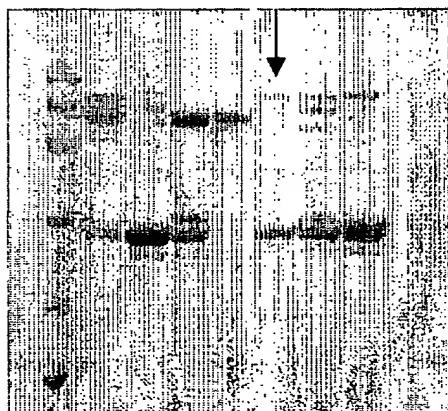
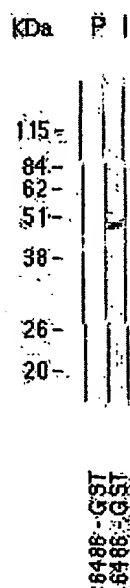


Fig. 117B



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FIGURE 118

FIG. 118A

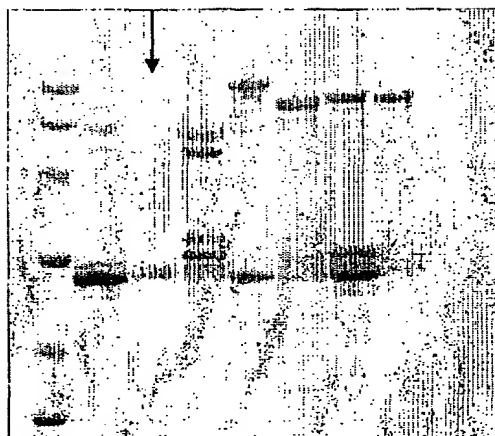
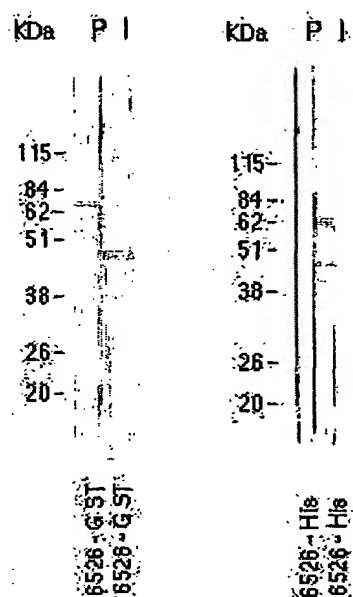


FIG. 118B



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FIGURE 119

Fig. 119A

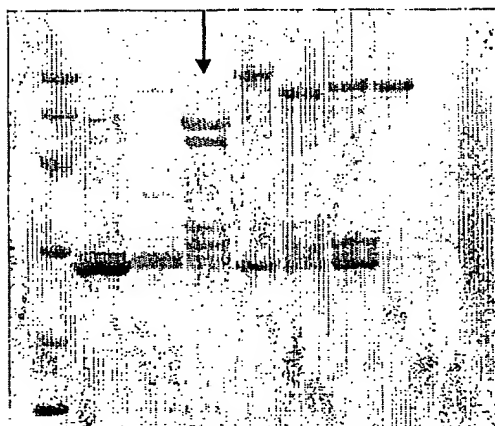
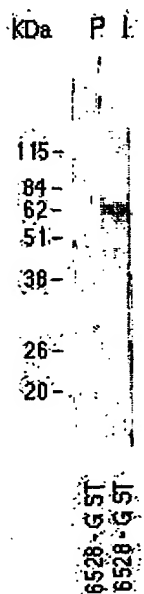


Fig. 119B



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FIGURE 120

FIG. 120A

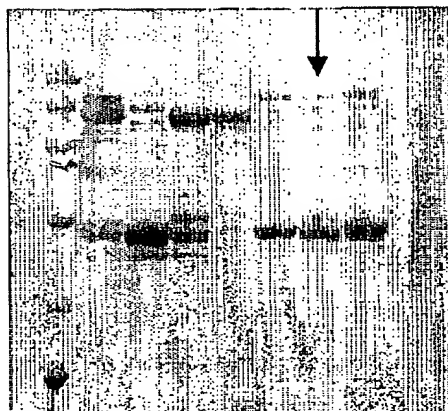
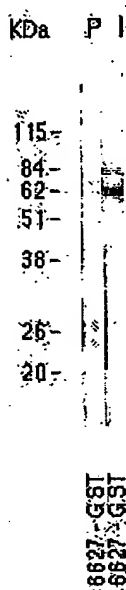


FIG. 120B



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FIGURE 121

FIG. 121A

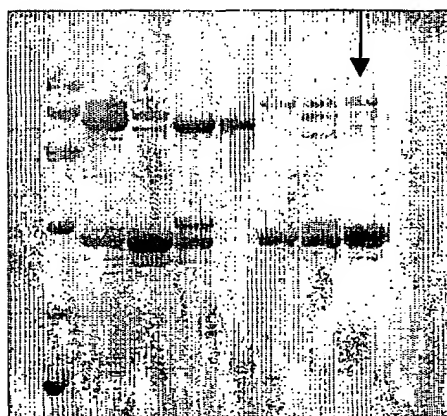
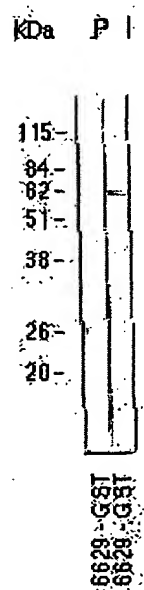


FIG. 121B



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FIGURE 122

FIG. 122A

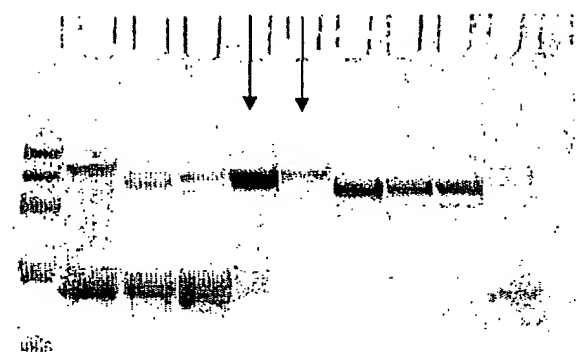
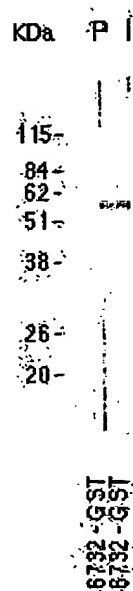


FIG. 122B



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FIGURE 124

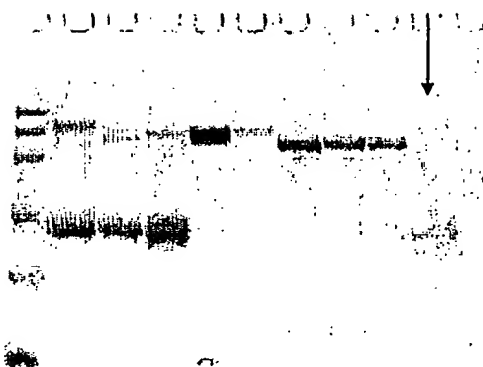


Fig. 124A

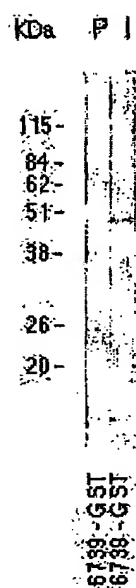


Fig. 124B

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FIGURE 125

FIG. 125A

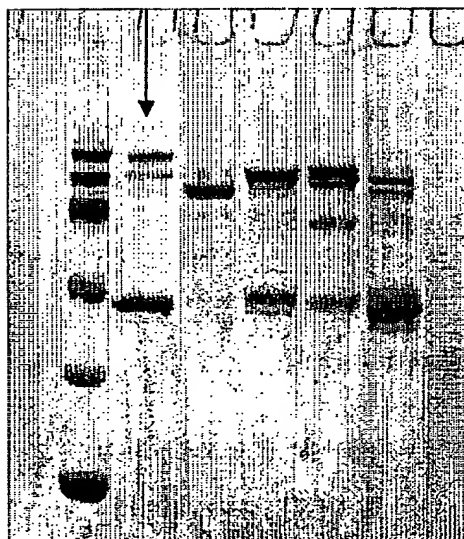
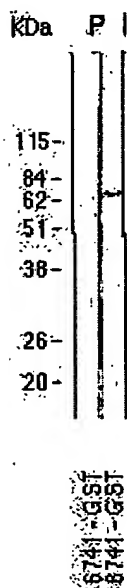
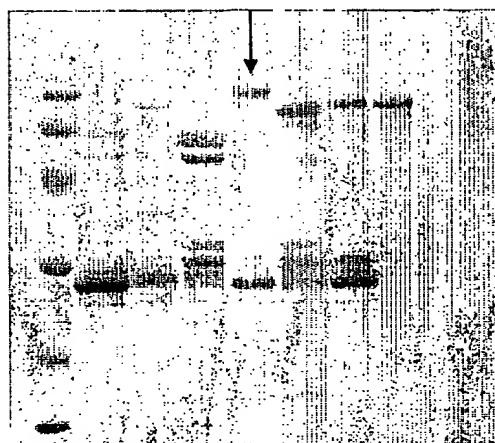
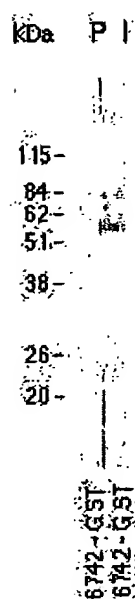


FIG. 125B



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FIGURE 126**Fig. 126A****Fig. 126B**

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FIGURE 127

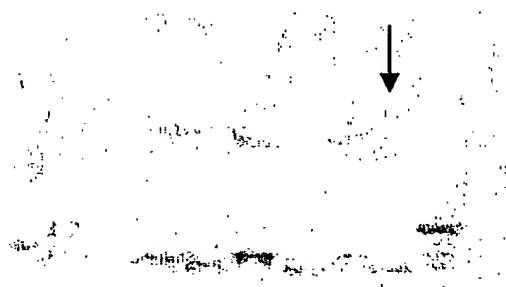


Fig. 127A

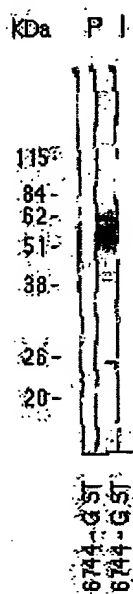


Fig. 127B

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FIGURE 128

Fig. 128A

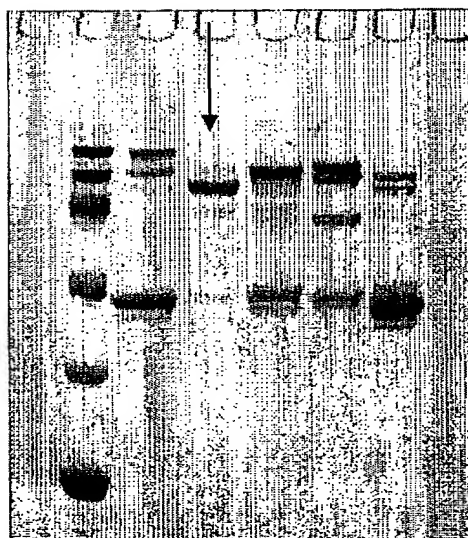
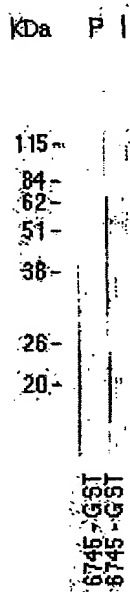


Fig. 128B



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FIGURE 129

Fig. 129A

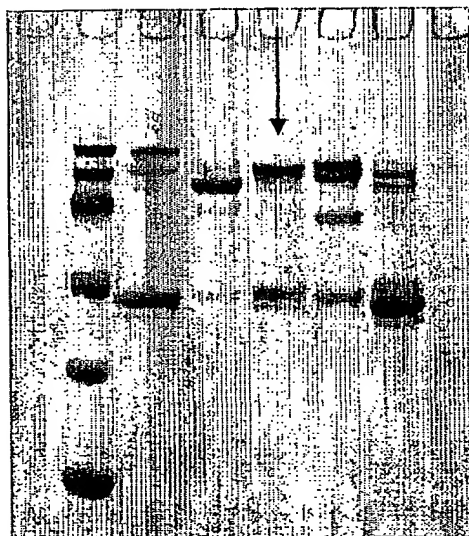
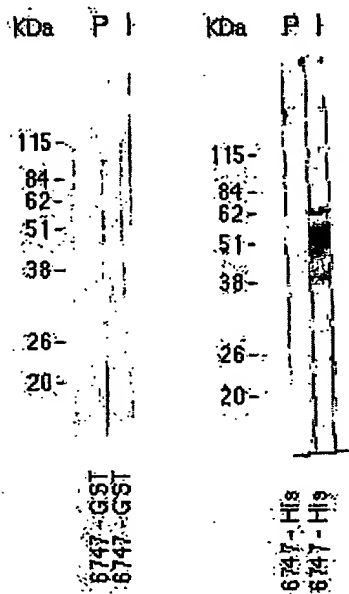


Fig. 129B



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FIGURE 130

FIG. 130A

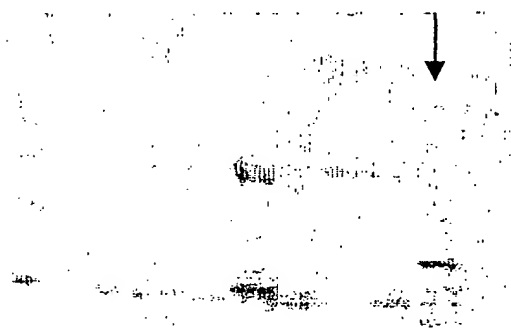
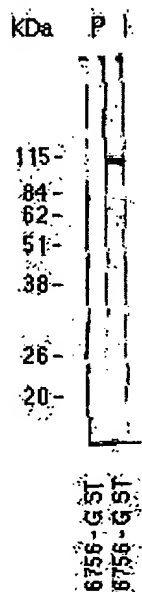


FIG. 130B



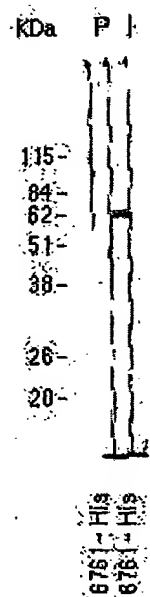
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FIGURE 131

Fig. 131A



Fig. 131B



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FIGURE 132

Fig. 132A

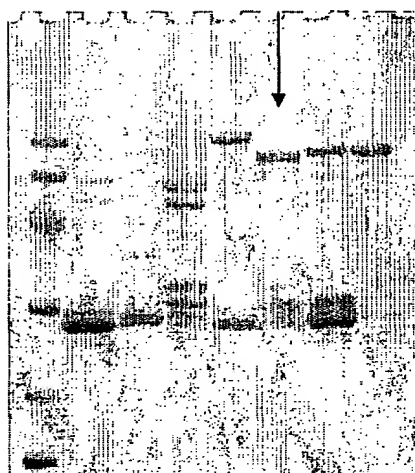
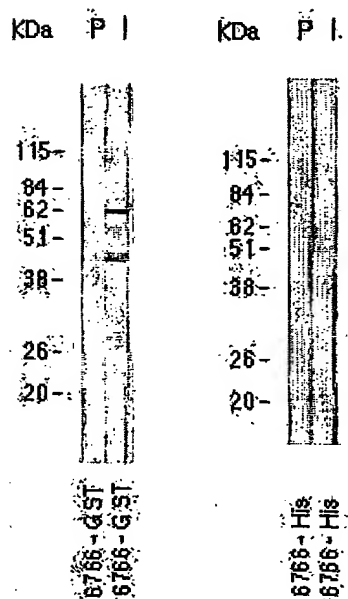


Fig. 132B



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FIGURE 133

Fig. 133A

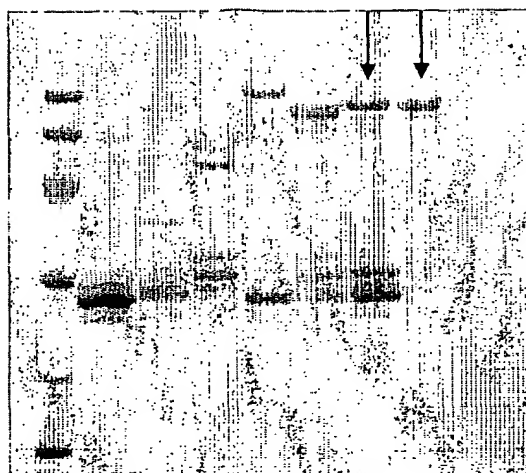
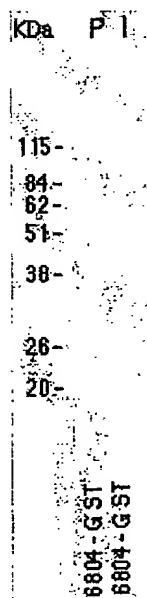


Fig. 133B



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FIGURE 134

FIG. 134A

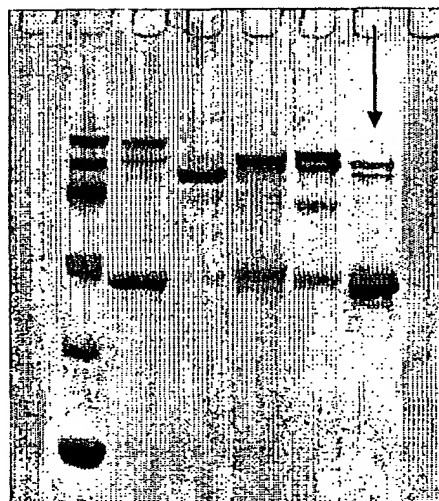
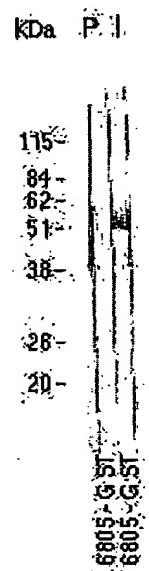


FIG. 134B



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FIGURE 135

FIG. 135A

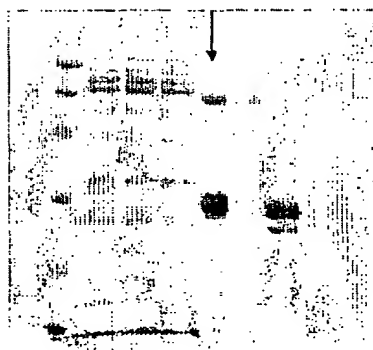
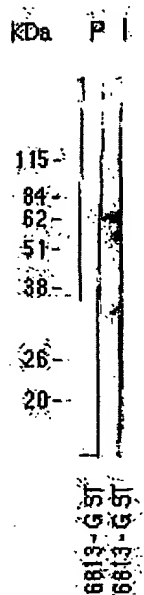
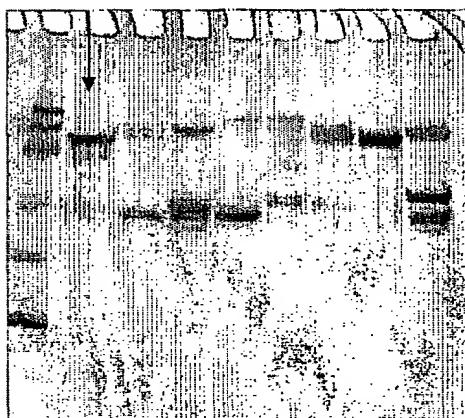
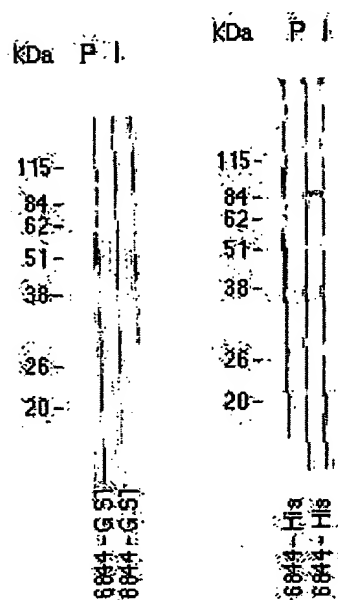


FIG. 135B



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FIGURE 136**Fig. 136A****Fig. 136B**

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FIGURE 137

FIG. 137A

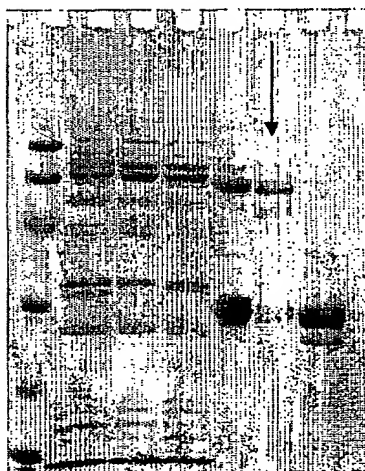
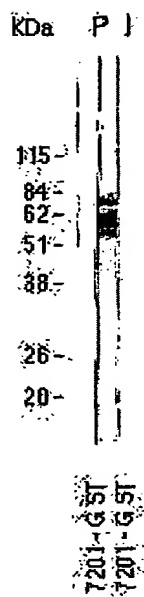


FIG. 137B



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FIGURE 138

FIG. 138A

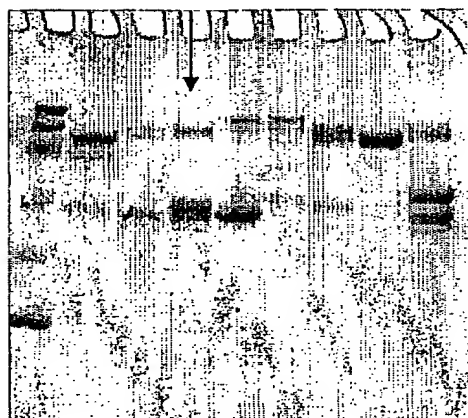
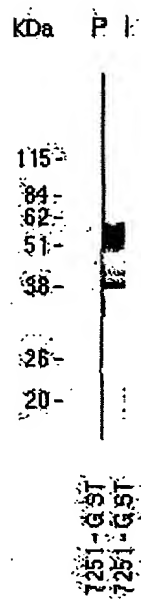


FIG. 138B



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FIGURE 139

Fig. 139A

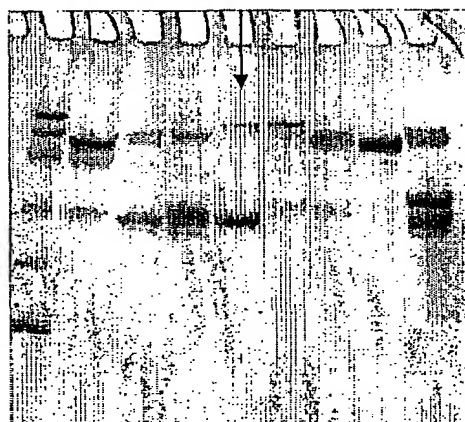
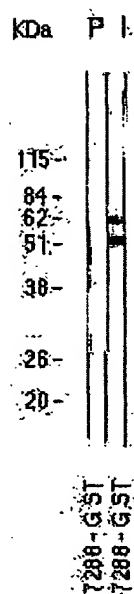


Fig. 139B



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FIGURE 140

Fig. 140A

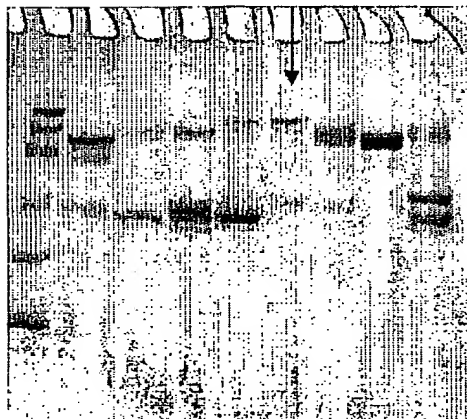
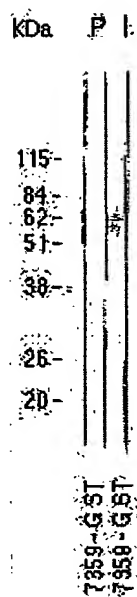
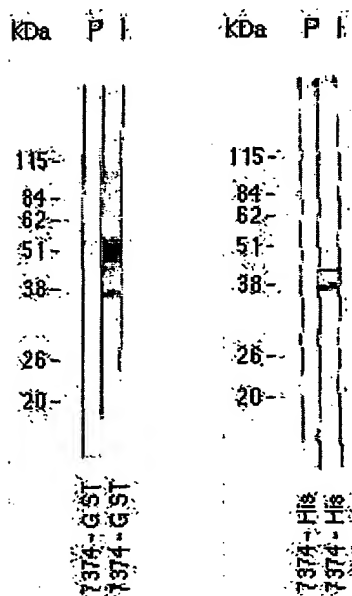


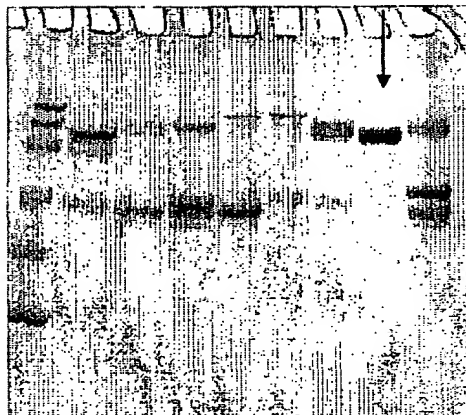
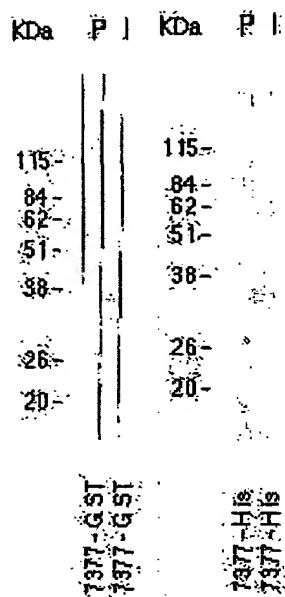
Fig. 140B



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FIGURE 141**Fig. 141A****Fig. 141B**

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FIGURE 142**FIG. 142A****FIG. 142B**

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FIGURE 143

Fig. 143A

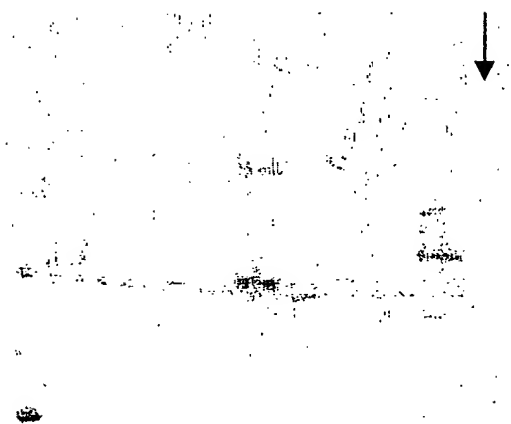
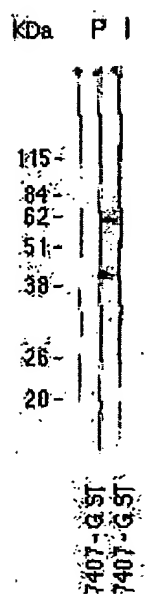


Fig. 143B



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FIGURE 144

Fig. 144A

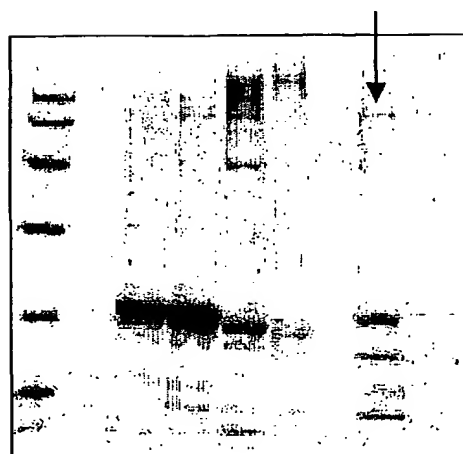
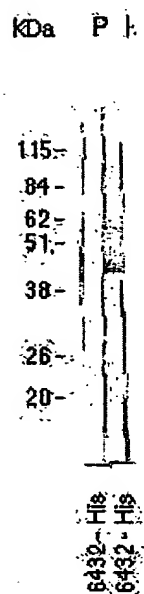


Fig. 144B



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FIGURE 145

Fig. 145A

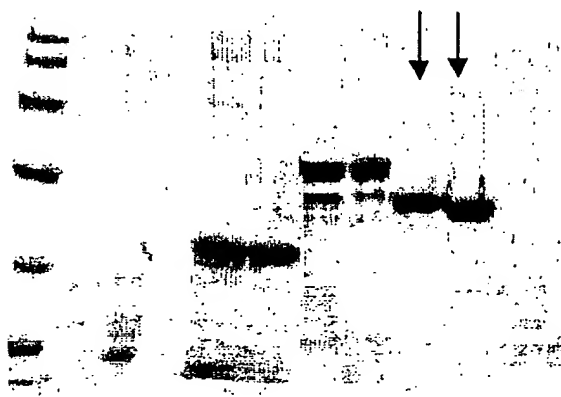
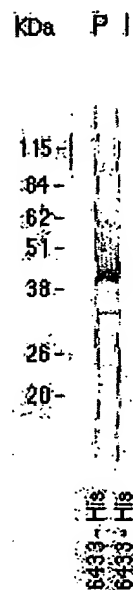


Fig. 145B



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FIGURE 146

FIG. 146A

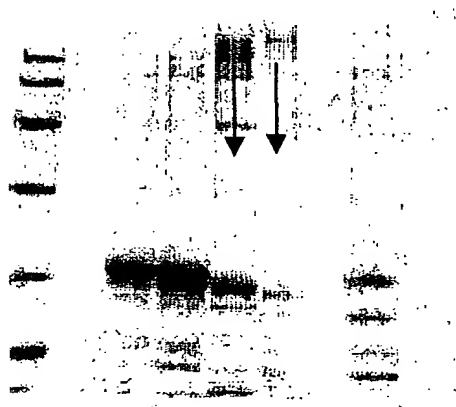
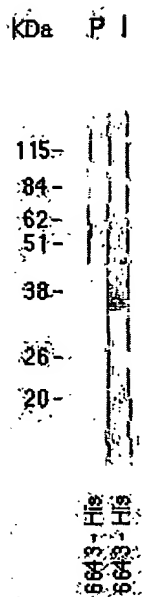


FIG. 146B



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FIGURE 147

FIG. 147A

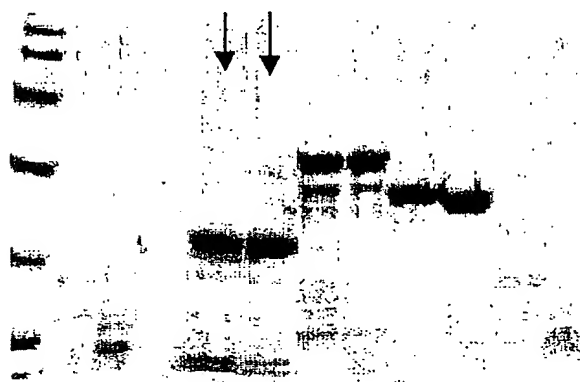
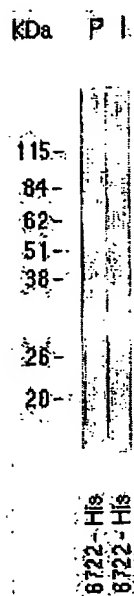


FIG. 147B



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FIGURE 148

Fig. 148A

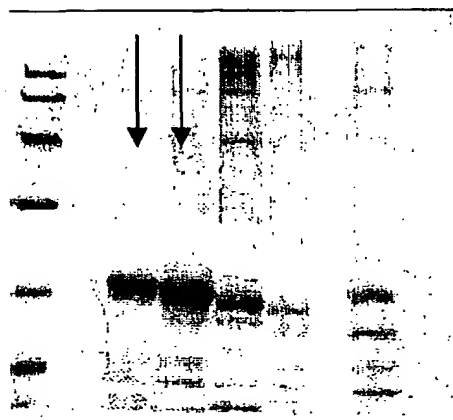
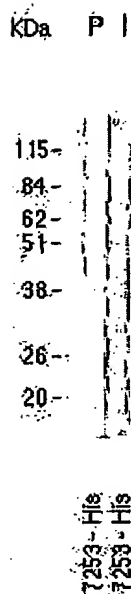


Fig. 148B



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FIGURE 149

Fig. 149A

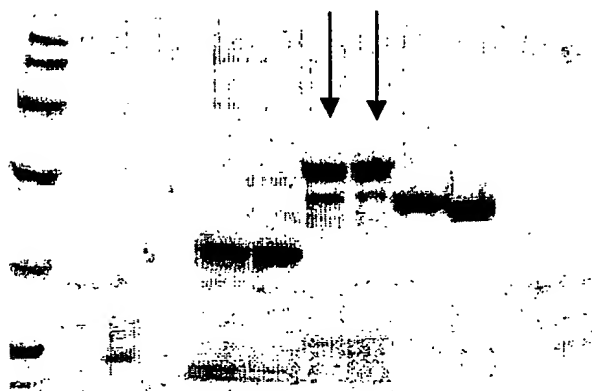
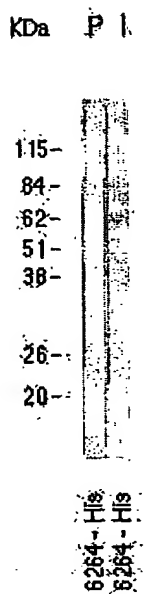


Fig. 149B



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FIGURE 150

Fig. 150A

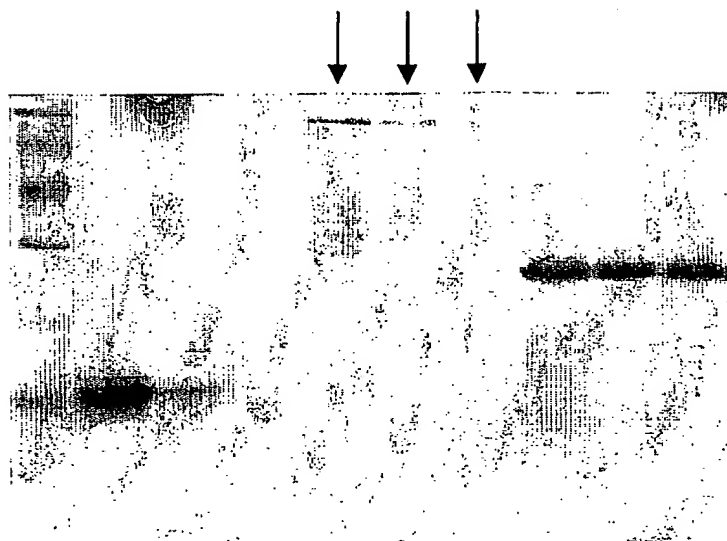
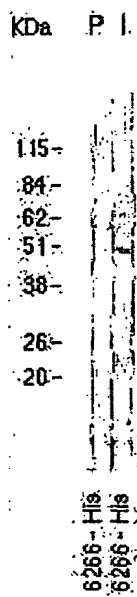


Fig. 150B



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FIGURE 151

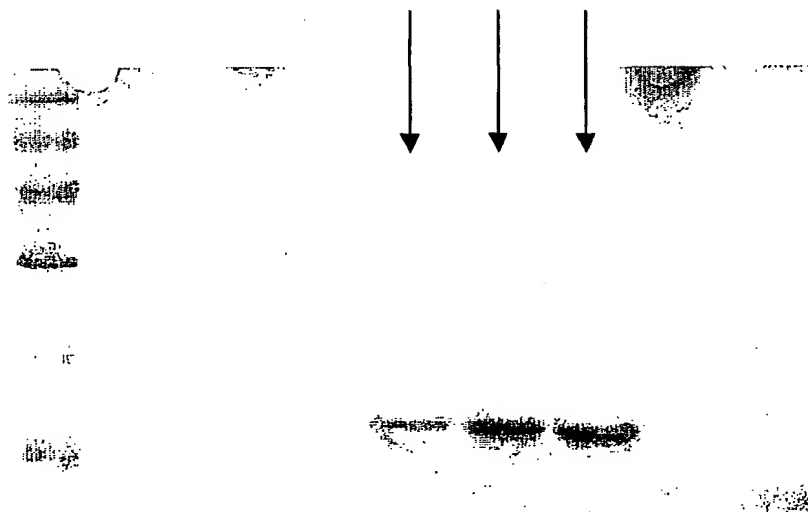
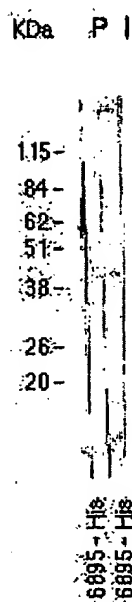


Fig. 151A

Fig. 151B



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FIGURE 152

Fig. 152A

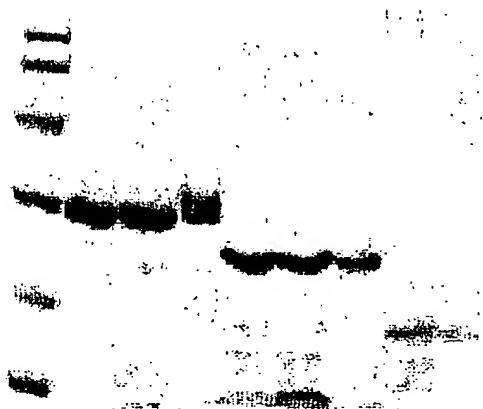


Fig. 152B

kDa P I

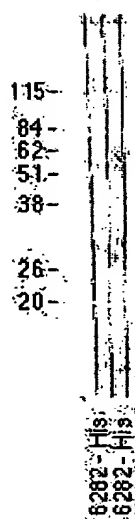
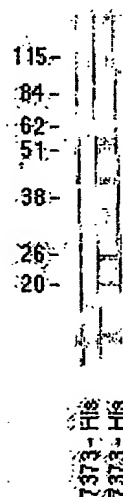


FIGURE 153

kDa P I



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FIGURE 154

FIG. 154A

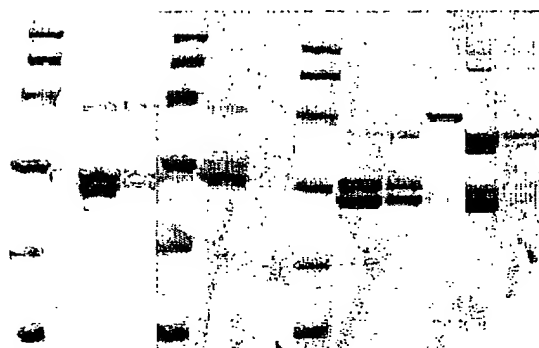


FIG. 154B

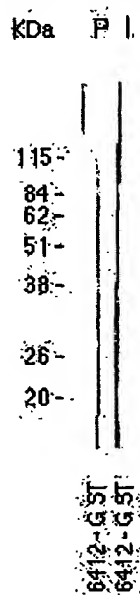
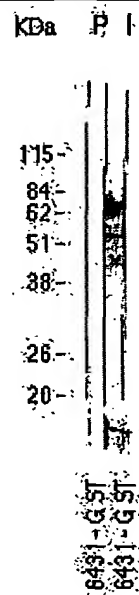
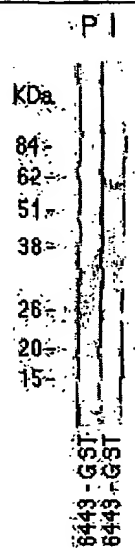
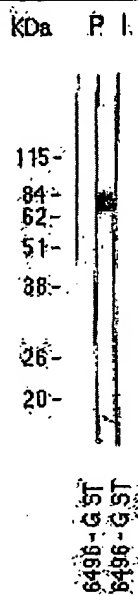
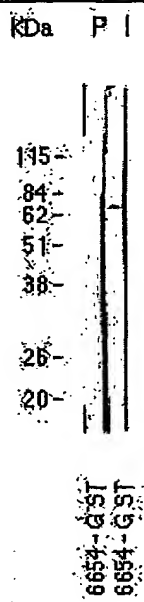


FIGURE 155



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FIGURE 156**FIGURE 157****FIGURE 158**

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FIGURE 159

Fig. 159A

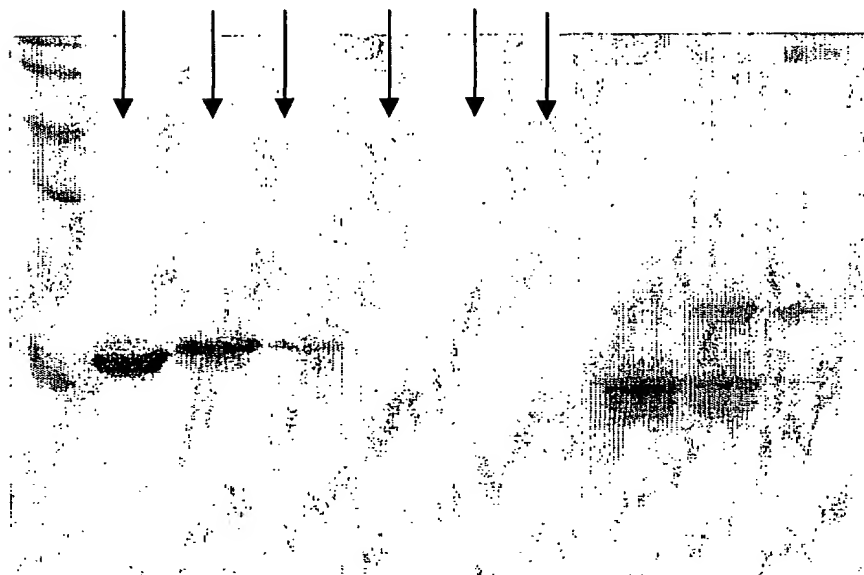


Fig. 159B

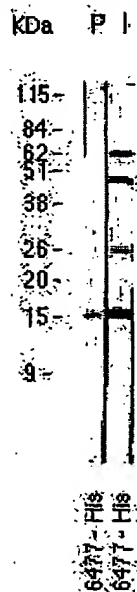
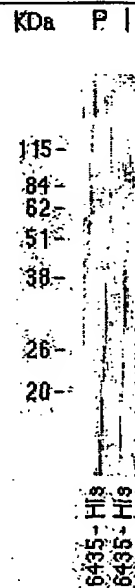


FIGURE 160



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FIGURE 161

Fig. 161A



FIG. 161B

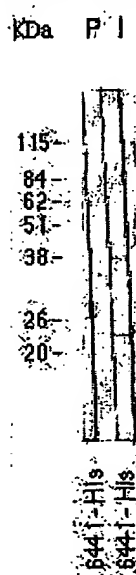


FIGURE 162

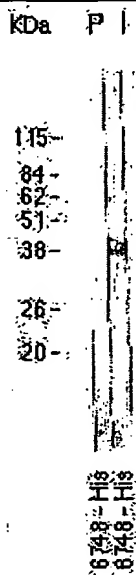
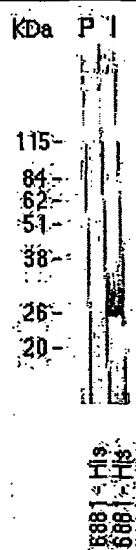


FIGURE 163



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FIGURE 164

Fig. 164A

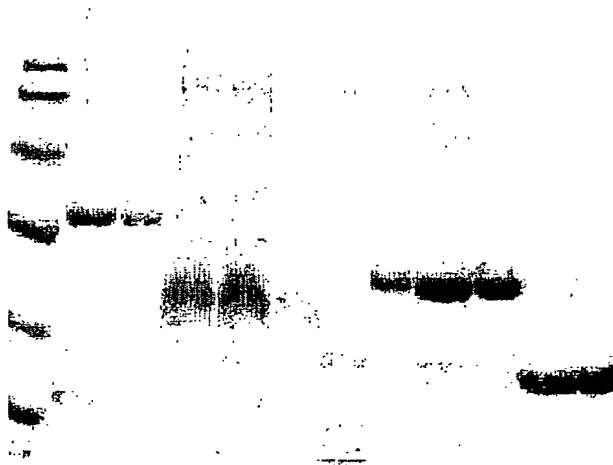


Fig. 164B

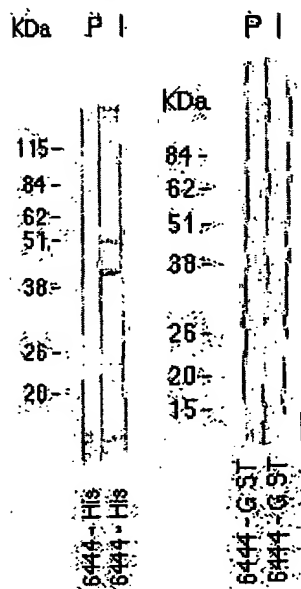


FIGURE 165

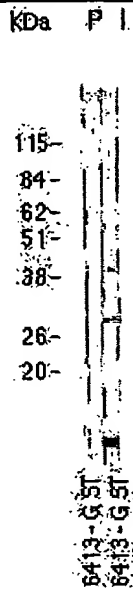
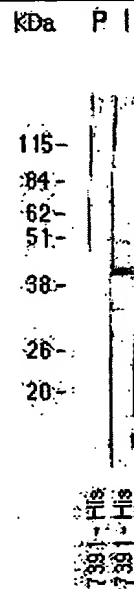
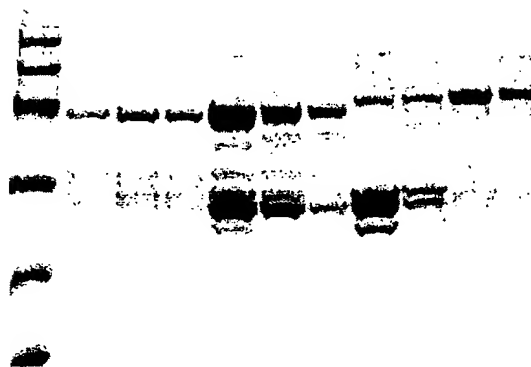
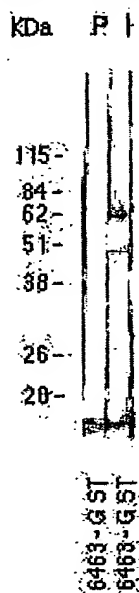
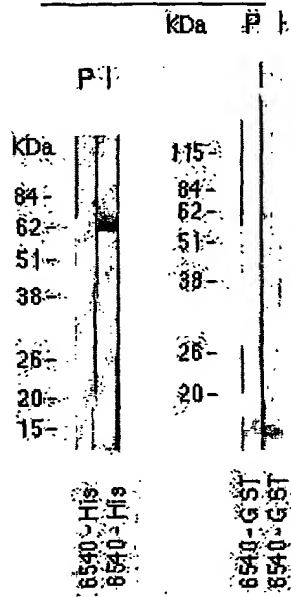


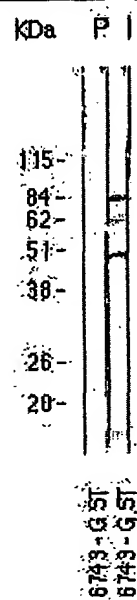
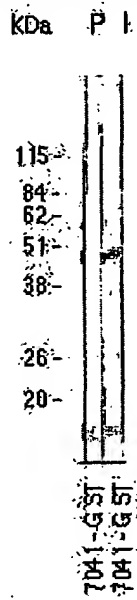
FIGURE 166



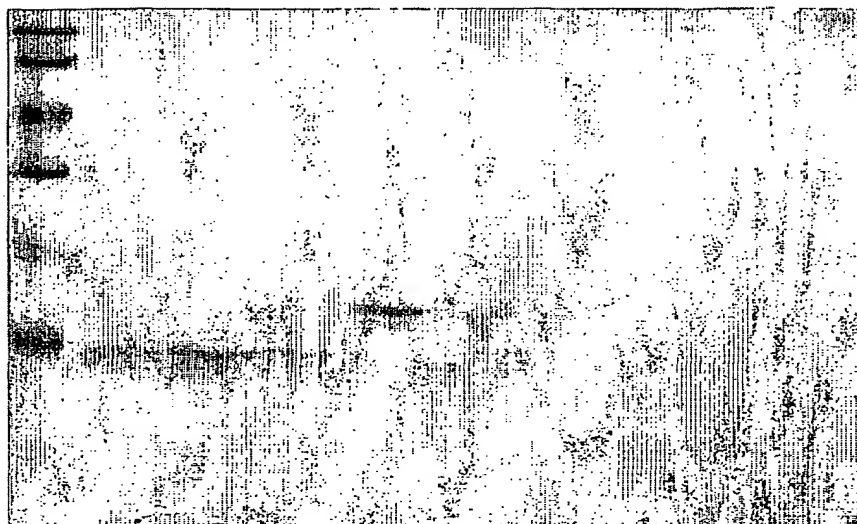
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FIGURE 167**FIG. 167A****FIG. 167B****FIGURE 168**

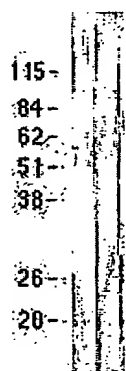
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FIGURE 169**FIGURE 170**

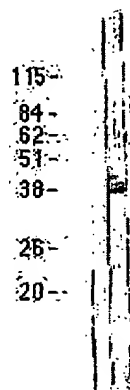
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FIGURE 171**FIG. 171A****FIG. 171B**

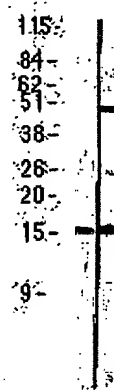
kDa P I

6632-His
6632-His**FIGURE 172**

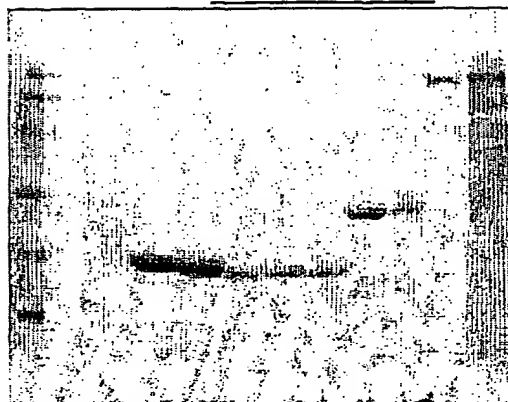
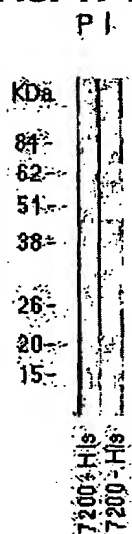
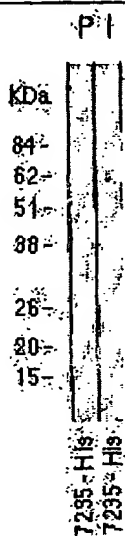
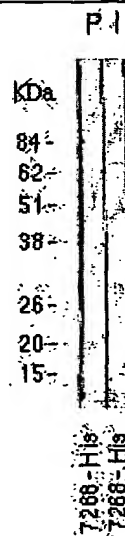
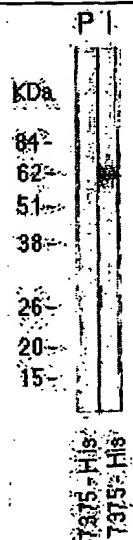
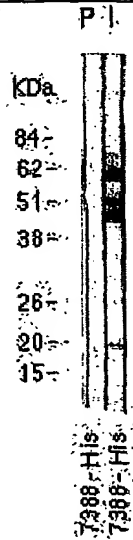
kDa P I

6748-His
6748-His**FIGURE 173**

kDa P I

6497-His
6497-His

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FIGURE 174**Fig. 174A****FIG. 174B****FIGURE 175****FIGURE 176****FIGURE 177****FIGURE 178**

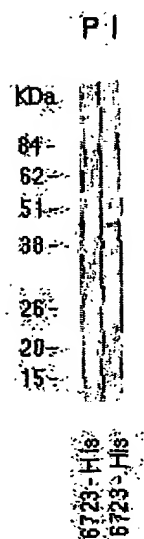
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FIGURE 179

Fig. 179A



Fig. 179B



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FIGURE 180

Fig. 180A

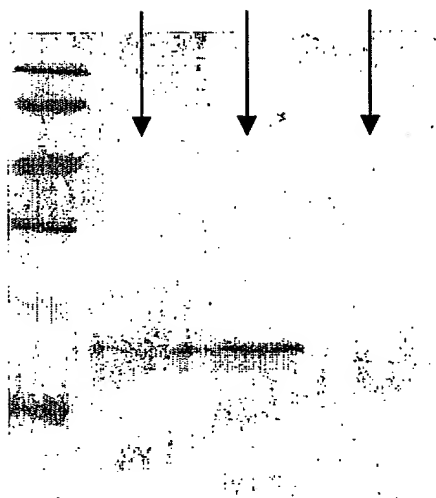
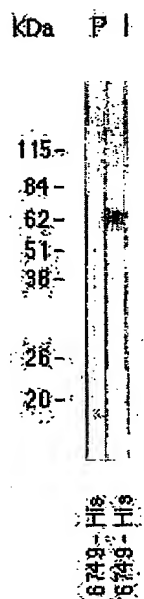
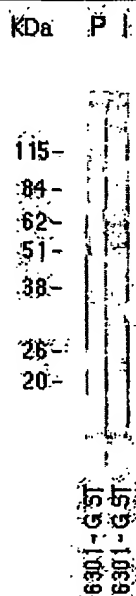
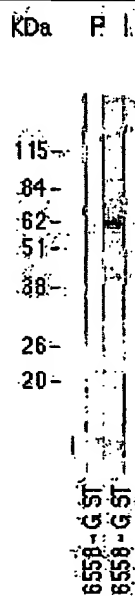
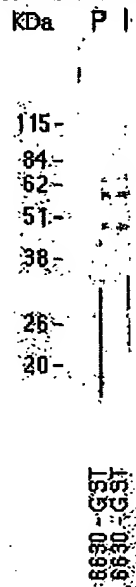
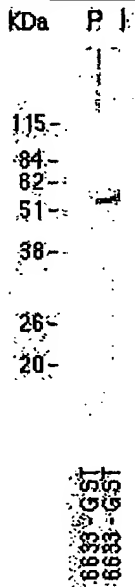


Fig. 180B



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FIGURE 181**FIGURE 182****FIGURE 183****FIGURE 184**

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FIGURE 185

kDa P I

115-

84-

62-

51-

38-

26-

20-

GST
GST
GST

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FIGURE 186

FIG. 186A

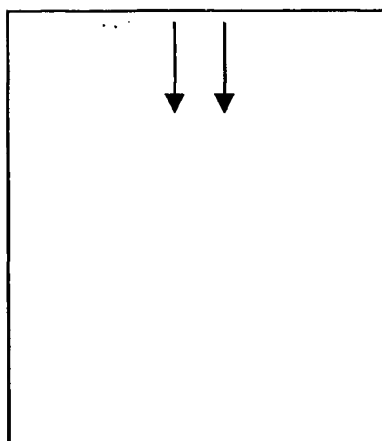
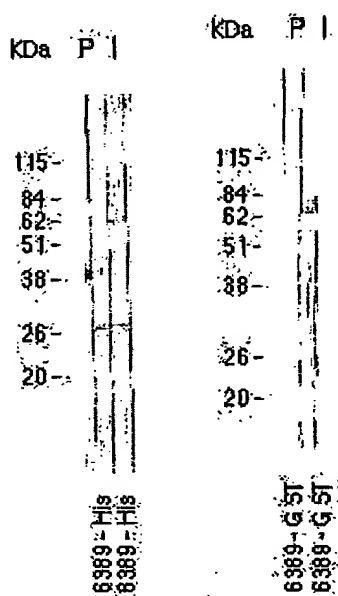


FIG. 186B



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FIGURE 187

Fig. 187A



KDa P L

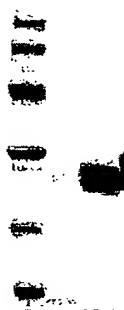
Fig. 187B



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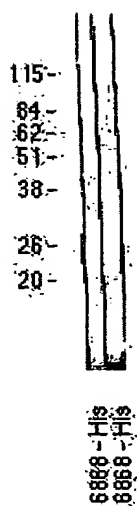
FIGURE 188

FIG. 188A



KDa P. I

FIG. 188B



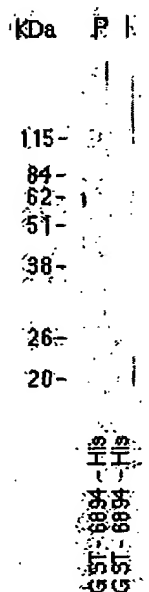
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FIGURE 189

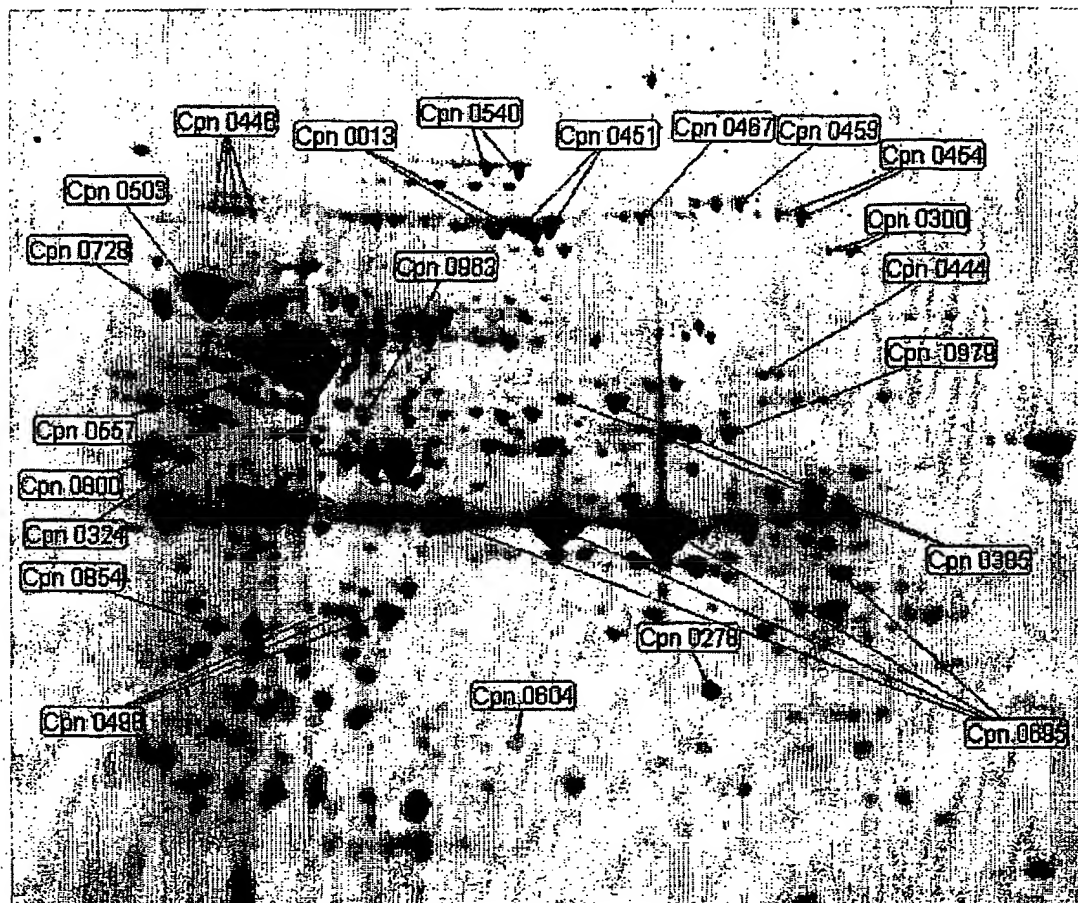
FIG. 189A



FIG. 189B



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FIGURE 190**FIGURE 191**

SVIVG.VSTNSEHRYHAFQYADGQMVDLGTLCGPESYAQGVSGDGK
 KVIVG.HSTRDGEYRAFKYVDGRMIDLGTLCGSASFAGVSDDGK
 KVIVG.RSETYYGEVHAFCHKNGVMSDLGTLCGSYSAAKGVSAATGK
 KVIVG.WSTTNNGETHAFMHKDETMHDLGTLCGGFSVATGVSAATGK
 TIIIVGSMESTITRKTTAVKVVNNVPTYLGTLCGDASTGLYISGDCT

